FINAL REPORT

Developing Functional Parameters for a Science-Based Vehicle Cleaning Program to Reduce Transport of Non-Indigenous Invasive Plant Species

SERDP Project RC-1545

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Statement of Non-Endorsement:

We do not endorse any of the products used during the course of this study.

Acronyms

AFPMB – Armed Forces Pest Management Board

ANOVA – Analysis of Variance

CONUS - Continental United States

CVWF - Central Vehicle Wash Facility

DOD – Department of Defense

ERDC-EL – Engineering Research and Development Center, Environmental Lab

GIS – geographical information system

GLM – Generalized Linear Model

GPS – global positioning system

HEMTT – Heavy Expanded Mobility Tactical Truck

IDARNG - Idaho Army National Guard

MSU – Montana State University

NIS – non-indigenous plant species

OCONUS - Outside Continental United States

OTA - Orchard Training Area

PLS - Palletized Loading System

SDTDC – San Dimas Technology and Development Center

US-FS – United States Forest Service

1.0 Abstract

Objectives

Transport and spread of non-indigenous species (NIS) is a worldwide concern at many management levels. Within the United States Presidential Executive Order 13122 directs Federal agencies to take whatever measures are available to minimize transport of non-indigenous species. The Department of Defense (DoD) and United States Department of Agriculture - Forest Service (US-FS) have responded to this order by performing vehicle cleaning under certain circumstances to avoid NIS transport, particularly plant propagules, to other sites. The goals of this project were to quantify the capacity of different vehicle types to spread plant propagules, develop occupancy or risk maps of target invasive and NIS, evaluate the effectiveness of mobile wash units to clean different vehicle types, and use this information to develop a prioritization protocol to help prevent NIS spread within and between DoD CONUS (Continental United States) and OCONUS (Outside Continental United States) installations.

Technical Approach

Adherence of soil to tracked, tactical wheel and civilian pattern vehicles and the effectiveness of mobile wash systems at removing soil was assessed in collaboration with the Forest Service. The rate at which seeds adhered to different types of vehicles was evaluated in collaboration with the military as part of training exercises. The rate of seed loss from a civilian pattern vehicle driven on paved and unpaved roads under wet and dry conditions was evaluated in a controlled experiment. Finally, sites were surveyed for invasive species, and the data used to create occupancy maps.

Results

All vehicle types gained considerable amounts of soil, with tracked vehicles gaining more than the wheeled vehicles. The vehicle wash units removed approximately 80% of available soil matter, and 77% of seeds were destroyed by containment procedures. This demonstrated that mobile wash units can be used as a preventative tool to reduce the risk of propagule spread. Significantly more seed adhered to tracked vehicles than wheeled vehicles under both wet and dry driving conditions. For wheeled vehicles there was a trend for more propagules to be gained on Humvees than tactical wheeled vehicles. And, as the proportion of distance Humvees travelled on unpaved and off-road increased, so did the number of propagules observed on the machine. In the more controlled study we found that once soil and seed are adhered to a vehicle, very little falls off under dry conditions when driving on paved or unpaved roads. In contrast, when the road surface is wet, soil and seed fall off, with the removal being more rapid on paved roads. These studies illustrate that vehicle type, road surface, and road condition need to be considered when evaluating the potential risk of vehicles becoming contaminated and spreading propagules.

Benefits

This project confirms that plant propagules adhere to, and can be spread by, vehicles, with these effects being amplified under wet conditions. Occupancy or risk maps of invasive NIS were generated and can be used to direct control practices and guide vehicle movement patterns

away from concentrations of target species and consequently minimize invasive plant species dispersal. These maps can be used in conjunction with mobile wash units to help prevent the spread of invasive plant species within and between military installations and adjoining lands.

2.0 Objectives

The overall objective of the project was to reduce the movement of non-indigenous plant species (NIS) propagules, within and between DoD CONUS and OCONUS installations. Different military vehicles were evaluated for their potential to transport NIS propagules using a commercially available vehicle washing unit that filtered and contained all of the waste material in the size range of NIS propagules. The effect of habitat, driving surface and moisture condition on the quantity of propagules transported was quantified on two sites over three years.

Specific project objectives

- 1. a) Evaluate the potential of different military (tracked, tactical wheeled and civilian pattern) vehicles for transporting NIS propagules, b) using 5 different vehicle washing units, c) to determine the degree to which the handling and disposal of propagules removed by vehicle washing units represents a potential infestation hazard.
- 2. Quantify the amount (species and their abundance) of propagules transported on three types of military vehicles (tracked, tactical wheeled and civilian pattern) in different habitats and environments.
 - Additional Approved Objective: Quantify seed loss from a vehicle on different road surfaces and under wet and dry driving conditions.
- 3. Develop NIS probability of occurrence or "risk" maps for the most prominent non-indigenous plant species at each test site. Determine environments more at risk of successful NIS invasion.
- 4. Develop a NIS containment and risk management prioritization protocol to reduce the occurrence of NIS and the chance of propagules being transported between and within installations in the continental U.S. (CONUS) and outside CONUS e.g. overseas deployment (OCONUS) based on the results from Objectives 1-3.
- 5. Evaluate and recommend improvements to the operation and design of the different vehicle washing units to improve their effectiveness, efficiency and containment capability. Develop specifications/classifications and standards for vehicle washing systems that are intended for remote and portable applications [Joint USDA Forest Service SDTDC (San Dimas Technology and Development) phase of the project].

3.0 Background

Invasion by non-indigenous plant species (NIS) is a global-scale problem that threatens the ecological integrity of native plant communities and ecosystems. NIS are introduced to areas by a variety of natural and anthropogenic means, and the number of anthropogenic dispersal events has increased in recent years (Hodkinson and Thompson 1997). Roads are often regarded as dispersal vectors for NIS due to secondary movement of NIS propagules to other areas by vehicles and, as such, provide more-disturbed environments that accelerate population expansion along the right of way (Veldman and Putz 2010). In Utah, species richness of NIS was 50% greater, and richness for native species was 30% lower, in sites next to paved roads than in sites next to 4WD dirt tracks (Gelbard and Belnap 2003). Cover of exotic species was also 50% higher at sites adjacent to paved roads than at sites adjacent to 4WD tracks (Gelbard and Belnap 2003). While roadside habitat may be conducive to weed infestations, it is also likely that vehicle dispersal explains part of the correlation found between NIS and roads.

Plant propagules (seeds and other reproductive parts) have been observed on vehicles (see Figures 1 and 2), but the number of studies is limited. Cars have been proven to be vectors for seed dispersal: seeds from 33 species were found on parked cars in suburban Belgium (Zwaenepoel *et al.* 2006), 37 species were found on cars in an English parking lot (Hodkinson and Thompson 1997), and 88 species were discovered on cars parked outside a national park in Northern Australia (Lonsdale and Lane 1994). Seed traps in a busy Berlin highway tunnel recorded an average seed rain of 635 to 1579 seeds/m²/yr with non-native seeds accounting for 50% of the 204 species identified (Von Der Lippe and Kowarik 2007). Cars travelling to logging landings in rural Bolivia were found to carry an average of 50 seeds/car on the tires and wheel wells, and 90 seeds/car on interior floor mats (Veldman and Putz 2010). Although these studies demonstrated that cars are vectors of long distance seed dispersal, none applied a dispersal curve or modeled their data because the distance the vehicles had driven before seeds were removed was unknown. Additionally, no studies of seeds carried on humans or their cars have been undertaken in North America (Pickering and Mount 2010).

Department of Defense (DoD) operations such as moving equipment, material, and personnel between sites at home and abroad, and driving off-road as well as considerable distances along unpaved roads, pose a high risk of transporting and depositing NIS within and between sites. Logging vehicles entering what was old growth tropical forest in Bolivia were credited with the introduction of NIS at logging landings within the forest (Veldman and Putz 2010). It is probable that military vehicles could have a similar impact. The amount of propagules adhering to vehicles is likely to be highest when driven off-road, less on unpaved roads and least on paved roads, plus, more propagules are likely to be collected by tracked or tactical vehicles than by civilian pattern vehicles, but there are no quantitative data to support either of these hypotheses. As germination and survival of NIS is often higher on disturbed land than on undisturbed land (Hobbs and Huenneke 1992) disturbances caused by operations on unpaved roads and off-road at many installations are likely to increase the chance of some propagules establishing and surviving, which in turn likely increases the number of new propagules which could be transported to other areas. Therefore, any methods and protocols which could prioritize control measures and reduce the probability of transporting and introducing NIS propagules would be beneficial to the DoD's NIS management responsibility and their efforts to develop an effective and mission-friendly response to Executive Order 13112.

There is currently "no standard washing time for any type of military vehicle. Vehicles are washed until they pass the standard however long this may take. This may vary based on the

number of operational power washers available for use, the number of personnel, and the number of wash racks" (Personnel communication with Dr. Cofrancesco of ERDC EL [Engineer Research and Development Center, Environmental Lab] and Dr. Peter Egan and LTC Jamie Blow of the AFPMB [Armed Forces Pest Management Board]).

The one criterion that appears to apply is stated within **UFC 4-214-03, 16 January 2004,** UNIFIED FACILITIES CRITERIA (UFC); **CENTRAL VEHICLE WASH FACILITIES.** This document is used to plan for the construction of new wash facilities, and is the DoD standard for this purpose. The washing rate, or throughput, is mentioned in two locations.

On Page 3-6, under the heading "Prewash," the following is stated: *Process Rate. In a tracked bath lane, six to ten tracked vehicles per hour can be washed. In a dual-purpose lane, ten to fifteen wheeled vehicles per hour can be washed. The amount of soiling will determine the actual number of vehicles that can be processed through the facility; the heavier the soiling, the slower the vehicles can be processed.*

Further, on Page 3-7, under the heading "Wash Stations," the following is given: Sizing with a prewash. When a bath prewash is provided, the number of wash stations should be rounded. If the maximum washing time is critical, between two and five per tracked vehicle bath lane. A process rate of 3 to 6 vehicles per hour at each wash station can be expected after the vehicles have been washed in the bath. Since all vehicles will not go through the prewash bath, calculations of lanes must account for longer wash times for these vehicles. A process rate of 2 to 4 vehicles per hour for large, odd shaped, or tandem units can be expected. A process rate of 4 to 10 vehicles per hour for small wheeled vehicles such as jeeps or ½ tons can be expected. Installations with a limited washing time or a large percentage of wheeled vehicles to wash will require more wash stations. In any case, the number of stations can be calculated using the processing rates for each type vehicle and its type.

In the next paragraph, the following is stated: Sizing without a prewash. The process rate for vehicles at the wash stations will depend on several factors, but usually will be between 1 and 10 vehicles per hour.

Thus, at DoD washing facilities within the US, units can spend as long or as short as they wish to wash a vehicle, but it appears that 6-15 minutes for wheeled and 6-20 minutes for tracked vehicles could be normal. As the washes are performed by the units themselves, not a contractor or service person, there are no records of the time taken.



Figure 1. Example of seed material accumulated on military vehicle.



Figure 2. Example of mud accumulated in the wheel well of a civilian vehicle.

4.0 Material and Methods

4.1 Objective 1

a) Evaluate the potential of different military (tracked, tactical wheeled and civilian pattern) vehicles for transporting NIS propagules, 1b) using 5 different vehicle washing units (in conjunction with the USDA Forest Service, San Dimas Technology and Development Center (SDTDC). 1c) To determine the degree to which the handling and disposal of propagules removed by washing represent a potential infestation hazard.

A number of different experiments were performed to address Objective 1 and are reported on below. The main series of experiments were performed at a California Department of Forestry and Fire Prevention Training Facility (CalFire)) in Ione, California during the summer of 2008. The experimental protocol for this summer long experiment was designed by Mr. Fleming, SDTDC, United States Forest Service (US-FS) after performing preliminary tests during the winter and spring of 2006-2007. This represented an in-kind contribution by the US-FS prior to the official start date of this project (May 2007). The CalFire site was located at 38° 21' 55" N, 120° 56' 24" S and the soil was classified in the Honcut series and is a coarse-loamy soil (Soil Survey Staff NRCS, 2009)

Several additional experiments to further elucidate effectiveness of wash units and the capacity of the wash unit process to destroy plant propagules were performed during 2007 and 2008 on Montana State University (MSU) property (45° 40'29" N, 111°09'14"W, 1423 m elevation) and at Orchard Training Area (OTA), ID (43° 17' 04" N, 116° 04' 46" S). For more detail about the technical aspects of the wash units please see Fleming (2008). The soil at MSU is classified as an Amsterdam-Quagle Silt Loam, and mainly Chilcott-Elijah and Purdam silt loams and Trevino Rock outcrop complexes at OTA (Soil Survey Staff NRCS, 2009).

4.1.1: Potential for vehicles to transport soil and plant propagules, and effectiveness of portable commercial wash units – Ione, CA.

The quantity of soil adhering to three different types of vehicles was assessed using vehicles that represented three types of military vehicles - tracked, tactical wheeled and civilian pattern (Objective 1a). Please refer to Figure 3 for pictures of specific vehicle types. The efficiency and effectiveness of five commercial portable wash units at removing the soil and other matter from these different vehicle types was also assessed (Objective 1b). The five commercial vehicle washing units that participated provided a representation of the range of available portable professional units that are for hire in the United States. Four of the units had undercarriage wash attachments and all had pressurized hand hoses. Water volume and pressure of the washing wands varied from 2 gallons per minute (gpm) @ 2,000 pounds per square inch (psi) to 9 gpm @ 1,200 psi. Under-carriage washers and other spray bars varied from 9 gpm @ 1,200 psi to 25 gpm @ 75 psi (see Fleming (2008) for more detail).

All vehicles were initially subject to a meticulous cleaning and close inspection prior to operating in the test area at CalFire, Ione, CA. Cleaning involved placing each test vehicle on an elevated wash rack, with tire removal as appropriate, and washing it thoroughly several times. The wash rack included a sump where all waste could be collected and contained for further evaluation. This was performed by the US-FS.



Figure 3. Examples of different vehicle types used for experiments. A = Humvee, B = M1A1 Tank, C = Personnel Carrier, D = Palletized Loading System (PLS), E = HEMTT Fueler, F = Bradley, G = ATV, H = Dodge Ram Pickup Truck, I = Class 3 Fire Engine, J = 4WD Truck.

After this initial meticulous cleaning, one tracked (bulldozer), two tactical wheeled (represented by Class 3 off-road fire engines) and three civilian pattern (two 4WD trucks and a SUV) vehicles were operated in the test area, becoming experimentally "contaminated" by driving a figure of eight course at a set speed of 16-24 km/hr (10-15 mph). The course was 1.38 km long and consisted of 638 m off-road and 742 m on unpaved and paved hard surface. The off-road section was scarified and contained a mud bog. The route was re-scarified daily and the bog was re-wetted between each vehicle run.

After driving the test loop, each civilian pattern and tactical wheeled vehicle underwent a five minute contractor wash followed by a "post-wash" by the US-FS. The vehicles then returned to the contamination area and the process was repeated. The drive and wash cycle was performed 18 times for each of the civilian pattern and tactical wheeled vehicle types for each wash unit contractor. The tracked vehicle underwent a modified contamination routine; the vehicle was transported to and from the off-road area on a trailer, and then driven around the off-road segment with the blades and rippers in use. Each contractor was allowed 60 minutes to perform

their wash of the dozer. The dozer then underwent a meticulous cleaning by the US-FS washing crew.

The soil and other debris collected from each vehicle type by each commercial wash unit was dried and weighed, as was the soil and other debris collected during the US-FS post-washes. At the end of each commercial wash unit test (18 cycles for each wheeled vehicle type and once for tracked), each vehicle was returned to the US-FS elevated rack for a meticulous inspection with wheels removed as appropriate, and additional cleaning performed to remove any remaining soil. This soil was dried, weighed and combined with the soil from the follow-up US-FS washes to determine the total amount of soil and other debris not dislodged by each of the commercial vehicle washing units. The waste for each vehicle and unit was collected and weighed in-situ. It was then air-dried (to less than 4% moisture) and reweighed until a constant weight was achieved. Any waste water from the system was further assessed using settling basins and filtered to 75 microns. Essentially, all solids greater than 75 microns in size were collected to determine the unit efficiency.

Evaluating the amount of soil and other matter adhering to the different vehicle types required approximately 5 days per vehicle washing unit. The experiment provided information on how much soil and other material can potentially be moved around on different types of vehicles. Including a wet area within the test area ensured that our estimates represent a "middle of the road" situation. In addition, by evaluating both the quantity of material that was removed by the initial pre-testing wash and the quantity that remained at the post-testing wash, we were able to determine the practical efficiency of each type of commercial washing system. The number of plant propagules in the soil collected by the different types of vehicle was not evaluated during this experiment as it was not possible to control the number of seeds available for each vehicle. However, we assessed the proportion of seeds destroyed by each vehicle wash unit's filtration process as described in the next section.

4.1.2 Soil and Seed Containment – Ione, CA

The aim of this experiment was to quantify the proportion of a known number of seeds that survive the containment process of a vehicle wash unit and germinate post-containment. These experiments were completed in conjunction with the experiments described above and used the same five commercial vehicle washing units. A preliminary set of studies were performed with one of the commercial wash units (Unit 4) in spring 2007 at MSU in order to develop a strict protocol for use in Ione and for Objective 2. The results of those studies are provided in Appendix A.

A homogenized mix of pasteurized sub-soil, sand and seeds was used. The subsoil and sand mix was used since filtration of finer particles (such as subsoil) would be more likely to compromise the filtration process and we wanted to evaluate the likelihood of that happening for each unit. Clogged filtering systems have been reported for vehicle wash units under US-FS fire camp conditions and this means that vehicles are not being washed properly when they need to be. (We did not observe any of the units filtration systems being compromised). Nine species were chosen to represent a broad range of seed shapes and sizes, *Avena sativa* being the largest and *Kochia scoparia* smallest. The full species list included: *Agropyron trachycaulum* (slender wheatgrass), *Avena sativa* (field oats), *Echinacea purpurea* (purple coneflower), *Fagopyrum sagittatum* (cultivated buckwheat), *Kochia scoparia* (kochia), *Linum usitatissimum* (cultivated flax), *Melilotus officinalis* (yellow sweet-clover), *Poa pratensis* (Kentucky bluegrass), and *Sinapis alba* (mustard). Seeds of each species (4772 per species) were combined with 80 kg of soil in a large rotating soil mixer and then slowly poured into a large plastic tub where large

quantities of water were added and mixed to suspend the soil and seed. The soil/seed mixture was then suctioned into the containment unit of each of the vehicle wash units via a submersible pump. Additional water was recycled into the plastic tub to ensure all the soil and seeds reached the containment unit. A 100 micron filter bag was placed over the recycled water outlet to catch any residual seed. The soil and seed sample was left in the containment unit overnight, as this is the common practice during field applications of these vehicle wash units. The following morning, the containment units were drained and cleaned thoroughly (using the standard protocol for each individual vehicle wash unit) and the resulting soil and seed samples were packaged into coolers with ice blocks and transported to MSU. The sample was mixed with pasteurized MSU mix (loam: Canadian Sphagnum peat moss: washed concrete sand in equal volumes) at an approximate 1:1 ratio and placed in flats in a greenhouse. The flats containing the soil mixes were watered daily and germinating and emerging seedlings were counted and removed twice weekly. The soil within the flats was disturbed weekly and the experiment terminated after 12 weeks. The temperature conditions in the greenhouse were set to 15 °C and 22 °C: night/day.

The methods used by the commercial wash units to contain and dispose of the soil and other waste under normal operating procedures were recorded but not formally assessed as part of this study.

4.1.3 Optimizing washing time and number

Total wash time and the number of washes will have an effect on how clean a given vehicle is when the washing procedures are finished. To evaluate how much soil and other debris was removed by i) washes of particular durations and ii) by successive washes of the same duration, controlled experiments were performed with one of the commercial wash units (Unit 4, Little Red Hen, LRH) also used in Ione. Identical 1 ton Dodge Ram pickup trucks (Dodge Cummins 24 valve turbo diesel) were used for these studies.

To evaluate the effect of wash duration on soil removal, the truck was washed for 1.5, 3, or 6 minutes, and replicated 4-6 times. To evaluate how the number of washes affected material removal from the truck it was washed for five 3 minute durations in spring 2007 and 2008. The experiments were performed at Montana State University in 2007 and at Orchard Training Area in 2008. Each truck was meticulously cleaned prior to the study by washing the vehicle with the vehicle wash unit until no soil or other matter could be seen on the chassis or body (10-20 minutes).

The truck was "dirtied" using the same procedure for all tests. A known amount of mud (40 kg of the same homogenized mix of sub-soil and sand used in the Ione, CA experiments) was applied to the underside of the truck using an air-pressured (2,000 psi) mud applicator (15 cm diameter by 3 m long PVC pipe, self-made). A tarp was laid underneath the truck to catch any mud that did not adhere to the truck and the truck was driven up onto ~ 2 m elevated wheel ramps. The mud on the tarp was collected and weighed wet and dry to determine the amount of soil applied to the vehicle. The vehicle was left stationary for ~ 12 hrs to allow the mud to dry prior to the washing sequence. At the end of each wash, soil material removed from the truck by the vehicle wash unit was collected, dried and weighed: the smallest filter size was 200 microns. The percent material removed by each wash was calculated.

For the 2008 experiment, six species (field oats, cultivated buckwheat, cultivated flax, yellow sweet-clover, Kentucky bluegrass, and mustard) were used as surrogate weed species, and 7285 seeds were used per treatment. The procedure used to germinate and count the seedlings was the same as Section 4.1.2. ANOVA (Analysis of Variance) with Tukey Correction

for Multiple Comparisons, was used to evaluate the statistical significance of any differences in the amount of soil material removed between single washes of various times (1.5, 3, or 6 minutes) and for the amount of soil/seed material removed between five successive 3 minute washes.

4.2 Objective 2

Quantify the amount (species and their abundance) of propagules transported on three types of military vehicles (tracked, tactical wheeled and civilian pattern) driven in different environments.

4.2.1 Transportation of propagules on different vehicles under field conditions

Field sampling was performed in collaboration with the 1-163rd infantry battalion of the Montana National Guard at Limestone Hills, MT (46° 19′ 44″ N, 111° 33′ 56″ S), in 2007 and 2009 and at the Orchard Training Area, Boise, ID, in 2008 following coordination with the IDARNG (Idaho Army National Guard). Both sites would be classified as sagebrush steppe, though the Limestone Hills training area has much greater topographic differences and juniper, mountain mahogany, whitebark pine, black sage as well as big sage vegetation types are present. We participated in field exercises on June 10-13 2007 (Exercise 1), June 18-20 2007 (Exercise 2), June 1-12 2008 (Annual training exercise), and June 3rd-7th, 12-14th and 19-21st 2009 (Exercise 3, 4, 5 respectively). The soil at Limestone Hills is classified as mainly Pensore and Crago stony complex with considerable slopes, and at OTA as mainly Chilcott-Elijah and Purdam silt loams and Trevino Rock outcrop complex (Soil Survey Staff NRCS, 2009).

The vehicles were driven, as per their mandate, along unpaved and paved roads and occasionally off road. Between 10 and 17 global positioning system (GPS) units (courtesy of Dr. Paul Ayers, University of Tennessee) were used for each exercise. In 2007, four GPS units were placed on HUMVEES (hereafter Humvees), four on 8-10 wheel personnel carriers and one each on all terrain vehicle (hereafter ATV) rangers for each of the two exercises. The rangers were the vehicles most likely to go off-road during the exercises. All GPS units were relocated at the end of Exercise 1; only 9 of 10 were located after Exercise 2. In 2008, six GPS units were placed on Humvees, four each on HEMTT (Heavy Expanded Mobility Tactical Truck) fuelers, Tanks (M1 -A1), and Bradleys, and three on palletized loading systems (hereafter PLS). Of these vehicles several were not in commission for the majority of the exercise resulting in data from six Humvees, four fuelers, four tanks, three Bradleys and one PLS. Over the course of three exercises in 2009, the GPS units were placed and recorded information on six tanks (M1-A1), five Humvees, five Bradleys, and 1 personnel carrier. All GPS units recorded the positions of each vehicle as it was driven around the area, enabling us to define where the vehicle was driven and on what type of surface (paved, unpaved or off-road), and the habitat types through which the vehicle passed etc. Obtaining the exact locations of vehicle maneuvers provided the capacity to estimate seed load per distance driven, and if this varied with road type. No vehicles that participated in our study were driven off-road at Limestone Hills in either year; a few were driven off road at OTA.

The vehicles used in the exercises were washed by Unit 4 above, a commercial vehicle wash unit, for approximately 5-6 minutes before they started one of the official exercises. In 2007 vehicles were washed at the end of each day of the exercise, while in 2008 and 2009 it was only possible to wash them at the end of the whole exercise due to timing constraints of the battalion. The wash process of the last exercise in 2009 with the Bradleys had to be removed

from further analysis due to lack of adherence to the correct protocol. In all years the number of vehicles washed was lower than we had anticipated given the decommissioning of vehicles and changes in exercise schedules throughout the actual exercises; despite considerable attempts at logistics before and during all exercises these factors were beyond our control. Unfortunately, the reduction in working vehicles and access to wash them more regularly resulted in less replication than we desired. However, it should be noted that the battalions we worked with were generally very helpful and accommodating: other sites that had agreed to participate with the project had to decline in light of OCONUS and CONUS schedules.

Vehicles of the same type were washed together: under dry conditions civilian pattern vehicles (e.g. Humvees) were washed for 4-9 minutes, tactical wheeled (e.g. fuelers, PLS) were washed for ~12 minutes, and tracked vehicles were washed for 13-18 minutes. Under wet conditions the duration of washes at least doubled. The mixture of soil, seeds and other matter removed from the vehicle wash unit was placed in plastic bags within large coolers and transported back to MSU where each sample was mixed with the MSU pasteurized soil mix (containing a higher proportion of organic matter and sand), and placed in flats on a greenhouse bench. The transportation period was approximately 90 hrs (see Appendix A). The flats containing the soil mixes were watered daily and germinating and emerging seedlings were counted and removed on a weekly basis. The soil within the flats was disturbed at approximately monthly intervals or when a new flush of seedlings had been removed; the flats were also randomly relocated on the greenhouse bench at these time. The temperature conditions in the greenhouse were set to 15 °C and 22 °C, night/day, although there was some seasonal variation. Lighting followed the seasons, with occasional supplemental lighting for a specific reason such as to induce flowering. To address possible vernalization requirements, flats were moved to a cold, dark room (4 °C) for 8 weeks after 9 months. Flats were left in the greenhouse for 20 months before being removed, generally after no germination had occurred for several months. The data were summarized as total number of seedlings per species and per km driven, total number of species and also total number of native and NIS for each vehicle type and exercise as appropriate. Where sufficient replication existed ANOVA was used.

Additional Objective: Quantify seed loss from a vehicle on different road surfaces and under wet and dry driving conditions.

4.2.2 Seed loss from vehicles

Understanding the distance seeds can travel on a vehicle once adhered to the vehicle body is important when assessing the risk of NIS dispersal and developing NIS containment strategies. To quantify this process, two controlled experiments were designed and carried out, the first unsuccessful and the second successful.

The first attempt at establishing potential dispersal distances by vehicles was conducted at Orchard Training Area in 2008. A 1 ton Dodge Ram pickup was "dirtied" using the same procedure as described in Section 4.1.3. The same six crop species were used as surrogate weed species, and 7285 seeds were used per treatment. The vehicle was driven at 10-15 mph for three set distances 4, 17.4 and 32 km, plus a 0 distance control, before being washed by the wash unit for 6 minutes. This process was replicated 4 times. The soil and seed material from the wash was processed through the containment portion of the wash unit and the material recovered and packaged for transport. Wet weights of the recovered soil were collected. Soil moisture of subsamples was measured to determine the dry weight of the recovered soil. The soil and seed was contained and germinated in the greenhouse at MSU as described in Section 4.1.2 to

quantify the proportion of seed lost at each distance. In addition, all the soil and seed which did not remain on the vehicle during the application process was recovered, weighed and then germinated in the greenhouse using the same protocol. Environmental conditions proved hard to control and the process of adhering soil and seed to the chassis and recovering all the potential losses proved inherently variable. Thus, a second experiment was conceived to further control for these types of variation.

The goal of the second experiment was to quantify the loss of seed and soil-substrate from a vehicle driving various distances under different environmental and road surface conditions, but was performed under more controlled conditions. Known amounts of seed and soil were adhered to aluminum plates that were attached to, and then detached from, a vehicle at set distances. Seeds that remained on the plates were counted. Sections of paved and unpaved roads (henceforth referred to as transects) were driven between May and October, 2010 and May 2011 when the appropriate weather conditions existed. The paved transects were driven on a 143 km section of road from Bozeman, MT (45°40' N, 111°03' W) to Alder, MT (45°19' N, 112°06' W). The unpaved transects were driven on a 35 km section of improved gravel road on Dry Creek and Sixteen Mile Creek Roads beginning 13 km north of Belgrade, MT (45°46' N, 111°10' W). To achieve the desired distances, some sections of each transect were driven more than once. While this was less than ideal, sections of unpaved roads tend to be relatively short and it was not possible to find a longer transect in our area.

A retention frame was constructed and mounted to the chassis of a 2004 Ford Ranger 4x4 truck. Aluminum plates were fabricated and spray painted with the same textured rust resistant paint used on passenger vehicles. Plates were made to attach to the undersides, front and rear bumpers, and wheel wells of the truck. For the undersides and bumpers, 16 smooth featureless plates and 16 featured plates (each 0.1 m² and containing grooves and an overhanging lip) were fabricated to represent the underside of passenger vehicles. The plates were bolted to the frame underneath the truck (Figures 4 and 5). Wheel well plates were smaller (0.05 m²) and had a smooth surface to mimic the size of the normal wheel well mud flap and to fit within the space available. There were eight smooth wheel well plates, to provide two sets. These plates were also bolted to the vehicle but hung vertically.

A soil slurry containing a known amount of soil (55% sand, 26% silt, and 19% clay), water and seed was applied to the aluminum plates. The slurry for each plate contained 90 seeds total, 30 each of mustard, slender wheatgrass and wheat, chosen because of their low potential impact associated with their dispersal and spread on the landscape, and due to use in other experiments. At the start of each transect, 20 plates were attached to the frame underneath the vehicle. Two plates were mounted below the front bumper, two below the rear bumper, six each between the wheel wells, and one in each wheel well. To quantify the effect of distance driven, road surface and road moisture on seed-loss, four transects were driven in each road surface/condition combination (paved dry, paved wet, unpaved dry and unpaved wet). Initially, we drove one transect of each type to 128 km, but due to low loss rates we increased the transect length to 256 km for all future transect replicates. On each transect, plates (two underside, one bumper and one wheel well) were removed from the truck at each of eight distances (0, 4, 8, 16, 32, 64, 128, and 256 km) and placed in a plastic bag and box for transportation back to the laboratory. The location of the plates removed at each distance was randomized. As the number of slots on the frame was limited, some plates were attached after others were removed in order to achieve all distances. In addition, the '0' distances were performed independently, but all of the same procedures and protocols were observed. The truck averaged 65.97 km/hr on paved

transects and 42.73 km/hr on unpaved transects. Once in the laboratory, the plates were dried and weighed. The substrate was then scraped off the plates and run through a set of sieves to separate seeds from the soil mixture.



Figure 4. A wheel well plate being attached to the retention frame of a 4x4 wheel drive truck to which underside plates have already been bolted.



Figure 5. Soil and seed mixture on seed loss plates after being driven a specified distance on a wet road.

Two control measures were completed to account for experimental error due to these procedures. Control 1 tested for seed or soil loss due directly to the way soil and seed were applied to and removed from the plates. The soil and seed slurry (containing 90 seeds) described

above was applied to the plates and allowed to dry. The plates were then weighed, scraped, sieved and counted without attaching the plates to the vehicle. This was performed for each plate type (smooth, featured, wheel well), and replicated three times. A mean 88.9 (SD 1.3) seeds were recovered. Control 2 tested for seed loss due to transportation of plates and boxes to and from the transect. Soil and seed substrate was applied to the plates, which were then dried, placed in boxes and transported to, and back from the start of the transect without attaching them to the truck. The plates were then weighed and scraped, the substrate sieved and the remaining seeds counted. There were three replicates of each type of plate. A mean of 89.6 (SD 0.79) seeds were recovered. Analysis of variance was used to assess the effect of different variables to determine the most appropriate variables for the retention models.

Total seed recovered was converted to the proportion of seeds retained (*sr*) and used as the response variable for the analysis. Different dispersal/retention functions were selected from the literature and fit to the data. Non-linear least squares regression via the nls function in the R statistical package (R Development Core Team, 2010) was used to estimate the parameters for each model. Maximum likelihood and AIC were used to determine the best fitting model. More detail of the experimental procedures and analyses can be found in (Taylor *et al.*, in preparation).

4.3 Objective 3

Develop NIS probability of occurrence or "risk" maps for the most prominent non-indigenous plant species at each test site. Thus, environments more at risk of successful NIS invasion will be determined.

4.3.1 Plant species present in the seedbank versus adherence to vehicles

To evaluate if particular types of species are transported more by vehicles than others we assessed the number of species present in the seedbank at Limestone Hills and OTA. Soil seedbank samples were taken at Limestone Hills in October 2007 and September 2009, and at OTA in September 2009. Samples were taken within all of the mapped vegetation types that vehicles travelled through during exercises. Each sample consisted of 300 g of soil (10 bulked soil cores: 6 cm wide * 10 cm deep) that were taken within a 10 m² area. Samples taken on the road were taken to a depth of 6 cm due to difficulty penetrating any deeper, but the same total volume of soil was collected. Samples were taken to the MSU greenhouse, mixed in 2:1 ratio with MSU pasteurized soil mix and placed in flats. The flats were subject to the same watering and other greenhouse conditions including a cold period, and germination and recording procedure as described for all other soil/seed mixtures. Samples were kept for 20 months except those taken on the road and at OTA that were only kept for 12 months before termination because no germination had been recorded for two months.

At Limestone Hills three sets of samples were taken within each vegetation type: one set less than 5 m away from the road edge and one set 50 m away from the road edge. These sample locations were along the transect line traversed for above ground vegetation transects also performed as part of Objective 3. In addition, samples were taken from the center of the road (in 2009); sites were randomly chosen but based on roads used by the vehicles during the June 2007 and 2009 exercises. The vegetation on the road edge, defined as the area that is maintained by road maintenance procedures such as grading but infrequently driven, was assessed for above ground vegetation using a 10 m² area (4 m parallel and 2.5 m perpendicular to the road).

At the Orchard Training Area, 21 soil core samples were collected as above, and the above ground vegetation assessed from each of two distances from the road: 1 m and 5 m. The

road edge (1 m) was defined and assessed as above (4 m parallel and 2.5 m perpendicular to the road). The next sample assessed 2.5-6.5 m perpendicular to the road and 2.5 m parallel. This L shape sample procedure ensured that the 1 m sample point was always on the road edge and the 5 m one was on the vegetation alongside the road. Vehicles drove off-road within one area of the OTA. Four - 1 km transects starting on roads were randomly located within the area, and at 100 m intervals along each transect the above ground NIS vegetation was recorded. Soil samples were taken at the same intervals for two of the four transects. All soil samples were mixed with pasteurized soil and maintained using the above protocol. Assessment of vegetation included estimates of cover for the 10 most abundant NIS for a 10 x 10 m² plot.

We had proposed to quantify the impact of vehicle traffic on vegetation in different habitats/environments - as part of Drs. Ayers' and Howard's participation. However, despite considerable investment of time and visits to our sites they were not able to perform their field tests due to lack of availability of the required vehicles. The lack of these data did not impact our study greatly, because, in the Limestone Hills field sampling, no vehicles left unpaved or paved roads, and only a correspondingly small proportion of total mileage was performed off-road at OTA. Thus, our information of seed gain on vehicles and occupancy maps have still allowed for the development to field protocols as part of Objective 4.

4.3.2 Non-indigenous plant survey to create occupancy maps

To determine the NIS richness and diversity collected from the vehicle wash samples, we also required a survey of the NIS *in situ*. It was assumed that most of the NIS of interest to the environmental departments of the military and National Guard have been present in the areas of interest for considerable time and have reached a stable state, but are generally at low frequency on the landscape. Therefore, to estimate the NIS occurrence and distribution on the landscape we used a stratified random transects protocol (Rew *et al.*, (2005 and 2006)) that allowed a relatively large sample size to be collected at Limestone Hills. Transects were 500 m perpendicular to the road and 10 m wide. Transect start locations were randomly located on roads traversed by the vehicles in a geographical information system (GIS) prior to commencing field work. Additionally, transects were delineated to intersect the main vegetation habitat types that the vehicles drove through.

At the Limestone Hills training site, transect sampling was conducted during the summers of 2007 and 2009. During the period of September 10-18, 2007, and on September 1st, 2009, between 2 and 4 observers sampled for NIS along the previously defined transects. Two person teams walked a transect recording data in a GPS data dictionary. When a target NIS was intersected along a transect, the length of the patch was recorded along with some desired environmental data. In addition, Townsend High School students had collaborated with the National Guard Environmental Division to map areas to the east of Old Woman's Grave Road during the summers 2004-2010 with useable data for 2006-2009. Townsend High School is located at 46°19' N, 111°31' W, 1,172 m above sea level. It is approximately 1.4 km east of the Missouri River, and approximately 3.4 km southeast of the Limestone Hills training site. The address is 201 North Spruce Street, Townsend, MT 59644. All collected data were used to generate continuous NIS presence and absence data using extensions we have created in Arc View (Version 3.2, ESRI, Inc.) and an Excel (Microsoft Corp.) macro, and have more recently been programmed for use on the web (http://ippf.msu.montana.edu). The continuous data were generated at 10 m by 10 m resolution.

Data were analyzed using logistic regression and the best model was chosen using a stepwise AIC procedure. The coefficients from these regressions were used to generate

probability of occurrence maps for the key NIS, as well as for all NIS combined (see Rew et al. 2005 for details). Environmental and remote sensing data were used as independent variables. In order to generate predictive NIS maps of the area of interest, a GIS layer for the variable of interest for the entire area is required. Therefore, we used environmental data from digital elevation maps (10 m resolution), Landsat remote sensing imagery and a road layer made available by the installation. Probability of occurrence predictions and maps of the NIS were generated using coefficient values from the logistic regression (generalized linear model (GLM)) applied to continuous spatial variables in rasterized format, using an extension we wrote in Arc View and ArcGIS. The extension applied the logistic function to calculate the probability of plant occurrence given the odds of finding a particular species at each raster cell. The odds of finding the species are defined as the product of each variable in the model and its coefficient value, plus the beta intercept value. The value of each cell in the output raster, ranging from zero to one, represents the probability that the target species occupies the area defined by that cell.

At OTA we were able to perform a minimal NIS survey in late September 2009, limited due to field access issues. The sampling design for the OTA is described in more detail above, but briefly NIS cover data were collected in 44 (100 m^2) plots located along four 1 km transects, and in $21(10 \text{ m}^2)$ paired roadside plots . These data were evaluated for species dominance along the road and the off-road area used in training exercise.

5.0 Results and Discussion

5.1 Objective 1

5.1.1 Potential for vehicles to transport soil and plant propagules and effectiveness of portable commercial wash units – Ione, CA.

The evaluation of the five contracted vehicle wash units showed a relatively high efficiency with an overall mean of 79% of material removed for all units for the three vehicle types. However, the material removal efficiencies ranged from 62% to 94%, showing some variability between vehicle types and units (Figure 6), with no unit performing consistently best or worst. The removal pattern was similar between units for the two types of wheeled vehicles but changed for the tracked vehicle (Figure 6). When results from the different wash units were combined there was a trend for more soil to be removed in increasing order from the 4x4WD to fire engine to bulldozer but the standard deviations suggest there is likely no statistical difference (Figure 7). All units used their own trained crews and it was visually apparent that some crew members were more effective with the pressurized wash hoses/wands. Adequate training on how to use a pressure hose would improve efficiency in the field.

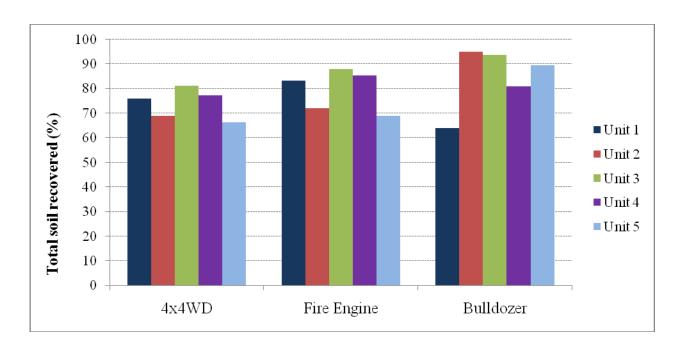


Figure 6. Total percentage of soil removed by the five commercial wash units for the civilian pattern (4X4WD), tactical wheeled (fire engine) and tracked (bulldozer) vehicles.

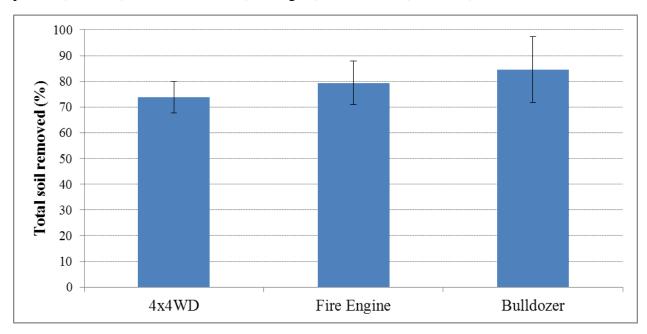


Figure 7. Percentage of total soil removed by all vehicle wash units combined for vehicle type. Error bars represent the standard deviation.

5.1.2. Soil and Seed Containment

It is possible that the containment and filtering process used by vehicle wash units destroys many of the seeds, and knowledge of this may help better guide disposal of this waste. The aim of this

experiment was to provide a standardized but conservative estimate of the percent of seed that are destroyed by the initial containment and filtering process of wash units. The normal operating procedure for the vehicle wash units is to empty the containment tanks out at the start of the day following vehicle washing. Therefore, soil and seed are left in the containment tanks for a minimum duration of 12 hours before the tanks are cleaned out and the contents filtered and water disposed of or recycled. To replicate this procedure we placed seeds of our nine species in the containment unit overnight and the following morning each vehicle wash unit used their own unit's cleaning protocol to remove the waste. The soil and seed waste was then bagged and transported to MSU where it was mixed with MSU soil mix, put in flats and left to germinate for a set period. The percentage of seed that survived the transportation protocol was determined in a separate experiment (see Appendix A for details), and these data were used to calculate the number of seed that were lost by each unit's containment protocol. That is, the percent of seed removed by the transportation procedure was subtracted from the total number of original germinable seed on a species basis. Seventy-seven percent of germinable seed were destroyed by the containment and filtering process. There were some visual differences between wash units but no further analysis could be performed due to lack of replication (Figure 8).

The percent of seeds destroyed was high (77%), but was likely a conservative estimate. Seeds were submerged overnight in the containment tanks, whereas under normal operating procedures waterlogged soil and seed mixture could be in the tanks for many more hours that would likely reduce the number of viable seeds further. For more complete information on the process rate, water use and cost please refer to Fleming's (2008) USFS Technical report 0851-1808-SDTDC.

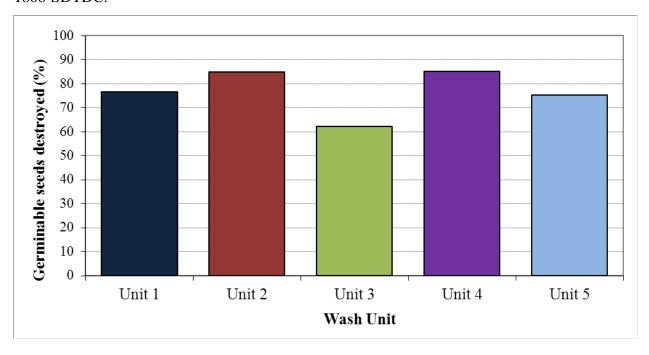


Figure 8. Percent of germinable seeds destroyed by the containment and filtering process of each of the vehicle wash units; mean loss was 77%.

5.1.3 Optimizing washing time and number

Longer duration single wash times resulted in more soil being removed than a single 1.5 minute wash (Figure 9). The average percent of total soil removed from the vehicle significantly differed (p=0.0002) between all wash times, and between all combinations at the p<0.04 level. The 6 minute wash removed the most soil and the 3 minute wash removed significantly more soil than the 1.5 minute wash (Figure 9).

The evaluation of successive 3 minute washes showed that an additional amount of material was removed upon each successive wash of the contaminated vehicle (p<0.001) in both 2007 and 2008 (Figure 10 a, b). Significantly more material was removed by the first wash (3 minutes) than any of the other washes for both 2007 and 2008, and in neither year did the last two washes representing 12 and 15 minutes of washing differ from each other. However, the second and third wash was significantly different in 2007 experiment but not 2008. These data suggest that a 3 minute wash removed most of the soil and other matter but an additional 3 minutes can further increase the amount of soil removed (Figure 10 a, b).

It should be noted that the amount of soil added to the truck was known for these experiments but the finest soil particles were not collected by the sieving process so the total amount recovered did not reach 100%. These finer particles were collected during the other experiments in settling tanks as described in 4.1.1, potentially accounting for the lower percent removed during these experiments as compared to the total percent removed for the experiment described in section 4.1.1.

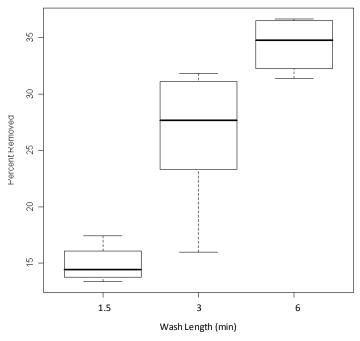


Figure 9. Percent soil material removed from a muddied truck during washes of different minute (min) durations using wash unit 4. The black line in the middle of the box represents the median; the box represents 50% of the data and the whiskers all the data. There were 4 replicates for the 1.5 and 6 minute wash and 6 replicates for the 3 minute wash. The amount of soil added to the truck was known but the finest soil particles were not collected by the sieving process so the total amount recovered did not reach 100%.

Seeds were added to the soil mix in the successive wash experiment in 2008. The number of seeds removed by each wash was determined by germination in the greenhouse. Significantly more seeds were removed by the first wash than all other washes (p<0.001). Seeds from the second wash were significantly more than the fifth wash (p=0.01); there were no further significant differences between successive wash combinations (Figure 11) nor amount (Figure 10b) of soil removed (Wash 1 and 2 were significantly different [p=0.06] with NS thereafter). The evaluation of the five commercial wash units suggested that wash units remove approximately 77% of the soil and other matter adhered to them. Further experimentation suggested that 3-6 minute wash duration is required to adequately remove dry adhered soil and seed, and that 3 but preferably 6 minutes should be the minimum wash time for wheeled vehicles.

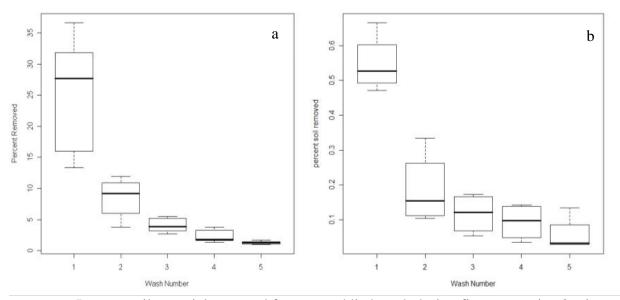


Figure 10. Percent soil material removed from a muddied truck during five successive 3 minute washes using the vehicle wash unit 4 a) in 2007, b) 2008. To determine the amount removed for any time interval the amounts should be summed. The black line in the middle of the box represents the median of 5 trials; the box represents 50% of the data and the whiskers all the data. The amount of soil added to the vehicle was known but the finest soil particles were not collected by the sieving process so the total amount recovered did not reach 100%.

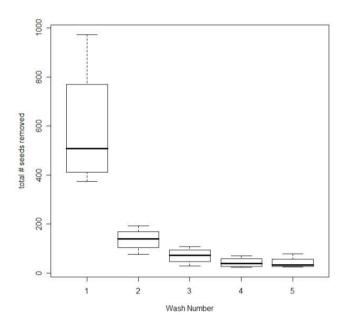


Figure 11. Total number of seeds removed from a muddied truck during each of the five successive 3 minute washes using the vehicle wash unit 4. To determine the total number of seeds removed for any time interval the amounts should be summed. The black line in the middle of the box represents the median of 5 trials; the box represents 50% of the data and the whiskers all the data.

5.2 Objective 2

5.2.1 Transportation of propagules on different vehicles under field conditions

5.2.1.1 Site 1 Limestone Hills

Both indigenous and non-indigenous species were recovered from the contained soil waste produced during post-exercise vehicle washes, indicating that these vehicles are capable of transporting seeds. A total of 61 species were identified during the course of the study (2007 and 2009) combined, of which 38 were native and 18 non-indigenous. Some species were grouped due to identification similarity and some could not be identified beyond genus due to lack of flowering under greenhouse conditions. Consequently, native or introduced status was not determinable for these species. In 2007, a total of 47 species were identified in the waste samples, of which 24 were native, 13 non-indigenous and a further 10 remained unidentified (Table 1). In 2009 a total of 31 species (18 native and 8 non-indigenous) were identified, with a further 4 unidentified (Table 1). Of the two 2009 exercises the first one was very wet, and the second dry providing a nice comparison in conditions. The native Verbena bracteata was the most abundant species in the vehicle samples, with the native grasses Sporobolus cryptandrus / Eragrostis pilosa and Hordeum jubatum also being fairly abundant: these species were also present in the soil samples.. In contrast the non-indigenous grasses Bromus inermis and Poa compressa were observed at higher abundances in the soil samples than vehicle samples (Table 1).

Overall, there was no statistical difference between the wheeled vehicle types in 2007 for the number of seeds per vehicle and km driven, with a mean of 4 seeds/km/vehicle gained and retained. However, there was a trend that Humvees gained and retained more seed than ATVs or personnel carriers (Figure 12). Only such basic comparisons were possible because the number of kilometers driven differed by vehicles, vehicle type, and exercise, as did the number of vehicles that completed each training exercise and the distance driven on paved or unpaved surfaces (Table 2). The vehicles primarily drove on unpaved roads, but during exercise 2 one Humvee and personnel carrier ((replicate (wash) 1) travelled only 35% and 17% of the total distance driven on unpaved roads respectively with the remainder driven on paved roads (Table 2). The number of seeds/km/vehicle was less than 1 (0.4) under these conditions for both vehicle types (Figure 12) compared with 4.9 seeds/km/vehicle for vehicles that drove more than 92% on unpaved surfaces. It should be noted that these data were collected under dry conditions in June prior to seed maturation in that year and thus probably represent some of the lowest seed gain rates likely to be observed during a season.

Table 1. Percent abundance of species observed, their native (n) or non-indigenous (i) status, and growth habit (forb (f), grass (g), shrub (s) and tree (t)) from vehicle waste samples of wheeled (Humvees and personnel carriers) and tracked vehicles (M1 tanks), under wet and dry conditions in 2007 and 2009 along with year totals. In additions percent abundance of soil samples taken on the road, less than 5 m from the road edge and 50 m from the road are provided. Total richness values are also provided.

	Vehicle wash												Seedbank				
Vehicle type/ seedbank location Conditions Year sample taken Species	n Status	Habit	Humvee Dry 2007	Carrier Dry 2007	ATV Dry 2007	Total Dry 2007	Humvee Wet 2009	Tank Wet 2009	Carrier Dry 2009	Humvee Dry 2009	Tank Dry 2009	Total Wet/Dry 2009	On road 2009	Off- road 5 m	Off-road 50 m		
Achillea millefolium	n,i	f	0	0	0	0	0	0	0	0	0	0	0	1	0		
Androsace septentrionalis	n	f	0	0	0	0	0	0	0	0	0	0	0	1	0		
Agropyron trachycaulus	n	g	4	1	0	5	0	0	0	0	0	0	0	0	0		
Agropyron spp.		g	0	0	0	0	2	5	0	3	1	4	0	0	0		
Amaranthus species retroflexus powellii	n	f	5	1	0	6	0	0	0	0	0	0	0	0	0		
Artemisia campestris	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Artemisia frigida	n	S	0	0	0	0	1	1	0	0	0	1	0	4	9		
Artemisia spp.	n		0	0	0	0	0	0	0	0	0	0	0	0	0		
Astragalus missouriensis	n	f,s	0	0	0	0	0	0	0	0	0	0	0	1	0		
Astragalus purshii	n	f	0	00	0	0	0	0	0	0	0	0	0	0	0		
Bouteloua gracilis	n	g	0	0	0	0	0	0	0	0	0	0	0	7	2		
Bromus inermis	n,i	g	0	0	0	0	0	0	0	0	0	0	0	9	42		
Bromus tectorum	i	g	0	0	0	0	0	1	0	0	0	1	3	0	0		
Carex stenophylla	n	g	0	0	0	0	0	1	0	0	0	1	0	9	1		
Chamaesyce maculata	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Chenopodium spp. (C. album, C. leptophyllum)	n,i	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Convolvulus spp.	i .	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cryptantha minima	n	f	1	0	0	1	0	0	0	0	0	0	0	0	0		
Descurainia pinnata/sophia	n,i	f	0	0	0	0	1	0	0	0	0	0	0	0	1		
Dyssodia papposa	n	f	2	0	0	2	0	0	0	0	0	0	0	0	0		

	Vehicle wash												Seedbank				
Vehicle type/ seedbank locati Conditions Year sample taken Species	on Status H	[abit	Humvee Dry 2007	Carrier Dry 2007	ATV Dry 2007	Total Dry 2007	Humvee Wet 2009	Tank Wet 2009	Carrier Dry 2009	Humvee Dry 2009	Tank Dry 2009	Total Wet/Dry 2009	On road 2009	Off- road 5 m	Off-road 50 m 2007		
Draba spp.		f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Epilobium ciliatum	n	f	0	0	0	0	0	0	0	0	0	0	0	1	0		
Euphorbia glyptosperma	n	f	0	0	0	0	6	5	0	0	1	5	0	0	0		
Festuca spp.		g	0	0	0	0	0	0	0	0	0	0	2	0	0		
Gnaphalium palustre	n	f	0	0	0	0	0	0	0	0	2	0	0	0	0		
Hackelia spp.	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Helianthus annuus	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Hordeum jubatum	n	g	2	1	0	4	3	9	0	11	8	8	0	16	17		
Juncus bufonius	n	g	0	0	0	0	0	0	0	0	2	0	0	0	0		
Juncus spp.	•	g	0	0	0	0	0	0	0	0	0	0	0	0	0		
Kochia scoparia	i	f	3	1	0	4	0	0	0	0	0	0	0	0	0		
Lactuca serriola	i	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Lepidium densiflorum/Gutierrezia		£ _	0	1	0	1	0		0	0	0	0	0	1	1		
sarothrae		f,s	0	1	0	1	0	0	0	0	0	0	0	1	1		
Linum rigidum	n	f	0	0	0	0	0	0	0	0	0	0	0	1	0		
Linum spp.		f f	0	0	0	0	0	0	0	0	0	0	0	6 0	0		
Lithophragma parviflorum	n i	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Malva neglecta Marrubium vulgare		f,s	0	0	0	0	0	0	0	0	0	0	0	0	0		
Medicago lupulina	i	f f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Melilotus officinalis	i	f	0	0	0	0	0	0	0	0	1	0	0	0	0		
Monolepis nuttalliana	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0		
Monroa squarrosa	n	g	0	0	0	0	1	1	0	0	0	1	0	0	0		
Nemophila breviflora	n	f	0	0	0	0	1	0	0	3	2	1	0	0	0		
Penstemon deustus		f,s	0	0	0	0	0	0	0	0	0	0	0	0	0		
Phacelia linearis	n	f	0	0	0	0	0	0	0	0	0	0	0	0	1		
Plantago patagonica	n	f	0	0	0	0	0	0	0	0	0	0	0	0	5		

				Vehicle	wash								Seedba	nk	
Vehicle type/ seedbank locatio Conditions Year sample taken			Humvee Dry 2007	Carrier Dry 2007	ATV Dry 2007	Total Dry 2007	Humvee Wet 2009	Tank Wet 2009	Carrier Dry 2009	Humvee Dry 2009	Tank Dry 2009	Total Wet/Dry 2009	On road 2009	Off- road 5 m	Off-road 50 m
Species	Status	Habit													
Poa compressa	i	g	0	0	0	0	0	0	0	3	0	0	2	0	0
Poa pratensis	i	g	1	0	0	2	0	0	0	0	0	0	0	29	14
Polygonum spp. (P. spergulariiform, P. aviculare and unknown)	n,i	f	0	0	0	0	0	0	0	0	0	0	0	0	0
Populus spp.	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculus spp.		f	0	0	0	0	0	0	0	0	0	0	0	0	0
Salix bebbiana	n	t,s	0	0	0	0	0	0	0	0	0	0	0	1	0
Salsola kali	i	f	2	0	0	2	0	0	0	0	0	0	0	0	0
Sisymbrium altissimum	i	f	0	0	0	0	0	0	0	3	0	0	0	0	0
Solidago missouriensis	n	f	0	0	0	0	0	0	0	0	0	0	0	0	0
Sporobolus cryptandrus/Eragrostis pilosa	n	g	7	2	1	10	10	27	0	10	8	22	0	2	2
Stipa viridula	n	g	0	0	0	0	0	0	0	0	0	0	0	00	0
Taraxacum officinale	n,i	f	0	0	0	1	0	0	0	0	0	0	0	0	0
Tetradymia canescens	n	s	0	0	0	0	0	0	0	0	0	0	0	1	1
Trifolium repens	i	f	0	0	0	0	0	0	0	0	0	0	0	1	1
Verbascum thapsus	i	f	1	0	0	1	6	4	0	3	5	4	0	4	0
Verbena bracteata	n	f	38	10	8	56	69	44	100	56	70	50	32	0	0
Yucca glauca	n	f,s	0	0	0	0	0	0	0	0	0	0	0	0	0
Total species richness			44	18	20	47	11	27	1	10	11	31	66	66	66
Native richness			23	11	10	24	10	18	1	5	8	18	1	15	12
Non-indigenous richness			13	6	9	13	1	6	0	3	2	8	2	5	4
Unknown spp. richness			8	1	1	10	0	2	0	2	1	4	1	1	0

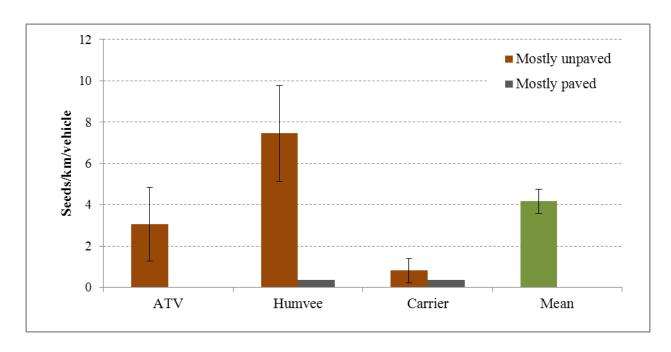


Figure 12. The number of seeds that adhered to different vehicle types per kilometer driven on predominately unpaved (<92 %) and paved (65% Humvee, 83% personnel carrier) road surfaces under dry conditions in June 2007 at a sagebrush steppe vegetation type at Limestone Hills, MT. The number of seeds/km/vehicle has been corrected for the proportion of seed destroyed by the vehicle washing and transport procedure described in Appendix A.

Table 2. The number of seeds gained on vehicles of different type when driven on paved and unpaved surfaces under dry conditions in June 2007 at a sagebrush steppe vegetation type at Limestone Hills, MT. Carrier = personnel carrier; ATV = all terrain vehicle with 6 wheels. Columns labeled "corrected" were adjusted for the proportion of seed destroyed by the vehicle washing and transport procedure described in Appendix A.

Exercise	Rep (wash)	Vehicle type	# veh- icles	Paved (Km)	Unpaved (Km)	Total (Km)	Seed/ vehicle	Corrected seed/ vehicle	Seed/Km /vehicle	Corrected Seed/Km/ vehicle
1	1	Humvee	5	23	267	290	61.8	95.6	5.3	8.2
1	2	Humvee	5	0	83	83	22.4	34.6	6.7	10.4
1	3	Humvee	5	0	65	65	24.4	37.7	9.4	14.5
2	1	Humvee	5	742	400	1142	10.4	16.1	0.2	0.4
2	2	Humvee	5	21	431	453	26.6	41.1	1.5	2.3
2	3	Humvee	4	0	157	157	12.0	18.6	1.2	1.9
1	1	Carrier	1	0	132	132	49.0	75.8	0.4	0.6
1	2	Carrier	1	0	132	132	91.0	140.7	0.7	1.1
2	1	Carrier	1	122	24	147	35.0	54.1	0.2	0.4
1	1	ATV	2	0	202	202	25.5	39.4	0.5	0.8
2	2	ATV	2	0	47	47	40.5	62.6	3.5	5.3

Fortuitously, exercises took place under wet and dry conditions during the exercises in 2009. Under wet conditions more waste was removed from tanks than under dry conditions resulting in 46 times more seed:165 seeds/km/vehicle under wet conditions compared to 3.5 seeds/km/vehicle under dry conditions (Table 3). The Humvee results were less dramatic with 21 times more propagules adhering to the vehicle under wet conditions - 10.5 versus 0.5 seeds/km/vehicle under wet and dry conditions respectively (Figure 13). These results clearly demonstrate that under wet conditions, tracked vehicles should not be driven on unpaved roads if there is any concern about moving soil and plant propagules.

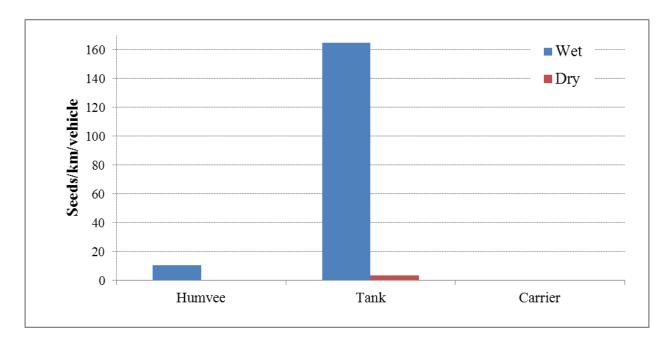


Figure 13. The number of seed that adhered to different vehicle types per kilometer driven predominately on unpaved road surfaces under wet and dry conditions in June 2009 in a sagebrush steppe vegetation type at Limestone Hills, MT. The number of seeds/km/vehicle has been corrected for the proportion of seed destroyed by the vehicle washing and transport procedure as described in Appendix A.

Table 3. The number of seeds gained on vehicles of different type when driven on paved and unpaved surfaces under wet and dry conditions in June 2009 in a sagebrush steppe vegetation type at Limestone Hills, MT. Carrier = personnel carrier; Tank = M1 tank. Columns labeled "corrected" were adjusted for the proportion of seed destroyed by the vehicle washing and transport procedure as described in Appendix A.

Exercise	Vehicle type	# veh- icles	Paved (Km)	Unpaved (Km)	Total (Km)	Seed/vehicle	Corrected seed/vehicle	seed/Km/ vehicle	Corrected seeds/Km/ vehicle
1	Humvee	2	0	77	77	262.0	405.1	6.8	10.5
1	Tank	4	0	85	85	2264.6	3501.7	106.5	164.7
2	Carrier	1	0	65	65	6.0	9.3	0.1	0.1
2	Humvee	3	4	172	176	20.3	31.4	0.3	0.5
2	Tank	2	0	92	92	105.0	162.4	2.3	3.5

5.2.1.2 Site 2 Orchard Training Area

As with site 1, both native and non-indigenous species were recovered in the contained waste, indicating that these vehicles are a significant mechanism in the transport of seeds. A total of 53 and 52 species were identified in the waste samples of wheeled and tracked vehicles respectively, of which 19 and 18 were native, and 25 and 22 were non-indigenous on wheeled and tracked vehicles respectively. Some species were grouped due to identification similarity and eleven could not be identified beyond genus due to greenhouse conditions, consequently native or introduced status was not determinable for these species (Table 4). The most abundant species was the non-indigenous species *Ranunculus testiculatus* for both wheeled and tracked vehicles, with the PLS (that travelled through a mud bog within the last 10 km before being washed) also having a large percentage of the native *Verbena bracteata* (Table 4). There were more forbs than grasses observed in the waste material, and only one shrub. The pattern of results was the same for the seedbank samples (Table 4).

Much longer distances were driven during this exercise than at Limestone Hills (Table 5). Most of the vehicles drove further on unpaved roads than off-road, although one set of Humvees drove mainly (97%) on paved roads, and one PLS (palletized loading system) travelled 104 km on unpaved roads but 6 km in a mud bog immediately prior to being washed. The seed load per vehicle and per km driven was lower than at Site 1, possibly as a consequence of the longer distances travelled or a possible "carrying capacity" of a vehicle although no statistical response was observed. The average number of seeds/km/wheeled vehicle was 0.32 excluding the PLS. The number of seeds/km driven for the PLS was 32, but it is likely that most of the seeds observed (3330) adhered to the vehicle in the last 6 km that were driven in a mud bog. There was a strong pattern to suggest that as the distance Humvees drove on unpaved and off-road, more seeds adhered to the vehicle as would be expected (Figure 14). There was no difference between the tracked vehicles – Bradleys and M1 tanks – but significantly more seed (1.3 seed/km/vehicle) adhered to them than wheeled vehicles (Figure 14).

Table 4. Percent abundance of species observed, their native (n) or non-indigenous (i) status, and growth habit (forb (f), grass (g), shrub (s) and tree (t)) from vehicle waste samples of all-wheeled (Humvees and Hemtt fuelers minus PLS), PLS (palletized loading system), all tracked (M1 tanks and Bradleys) vehicles and soil samples taken with 5 m of the road edge. Total richness values are also provided. The species in bold is listed as a noxious species in Idaho.

			Percent abundance							
Species/ Treatment	Status	Habit	All wheeled (-PLS)	PLS	All tracked	Seedbank < 5 m				
Agropyron spp.	n	g	0	0	0	0				
Alyssum spp.	i	f	0	0	0	0				
Amaranthus albus	i	f	0	1	5	0				
Amaranthus retroflexus/powellii)	n	f	0	. 0	1	0				
Arabidopsis thaliana	i	f	0	. 0	0	0				
Bromus tectorum	i	g	0	. 2	2	0				
Buchloe dactyloides	n	f	0	0	0	0				
Buglossoides arvensis	i	f	0	0	0	0				
Capsella bursa-pastoris	i	f	0	0	0	0				
Carex spp.		g	0	0	1	0				
Chenopodium album	n,i	f	0	0	1	0				
Chenopodium spp.		f	0	0	0	0				
Conyza canadensis	n	f	0	0	0	0				
Descurainia pinnata	n	f	0	. 0	0	0				
Descurainia sophia	i	f	0	0	0	10				
Descurainia spp.		f	0	. 0	0	0				
Draba verna	i	f	0	. 0	0	0				
Epilobium ciliatum	n	f	0	0	1	0				
Erysimum capitatum/repandum	n,i	f	0	0	1	0				
Erysimum repandum	i	f	0	0	1	0				
Galeopsis tetrahit	i	f	0	0	0	0				
Gnaphalium purpureum	n	f	0	0	0	0				
Hordeum jubatum	n	g	0	. 0	0	0				
Iris missouriensis	n	f	0	0	0	0				
Juncus ambiguus	n	g	0	. 0	0	0				
Juncus arcticus	n	g	0	0	0	0				
Juncus ensifolius	n	g	0	0	0	0				
Kochia scoparia	i	f	0	0	0	0				
Lactuca serriola	i	f	0	0	0	0				
Lepidium densiflorum	n	f	0	. 1	0	0				
Lepidium perfoliatum	i	f	1	0	0	12				
Malva neglecta	i	f	0	0	0	0				
Matricaria discoidea	i	f	0	0	0	0				
Medicago lupulina	i	f	0	0	0	0				

			P			
Species/ Treatment	Status	Habit	All wheeled (-PLS)	PLS	All tracked	Seedbank < 5 m
Myosurus spp.	n	f	5	2	1	0
Phleum pratense	i	g	0	0	0	0
Poa compressa	i	g	0	1	1	0
Poa pratensis	n,i	g	0	0	1	0
Poa sandbergii/juncifolia	n	g	0	0	1	0
Polygonum aviculare/minus	i	f	2	4	2	0
Polygonum aviculare	i	f	0	0	0	0
Portulaca oleracea	i	f	0	0	0	0
Potentilla spp.	n	f	0	0	0	0
Ranunculus testiculatus	i	f	86	38	75	77
Rorippa spp.		f	0	0	0	0
Salix hookeriana	n	s	0	0	0	0
Salix lasiandra	n	s	0	0	0	0
Salix spp. (not lasiandra or hookeriana)	n	s	0	0	0	0
Salsola kali	i	f	1	0	1	0
Silene latifolia/Lychnis alba	i	f	0	0	0	0
Sisymbrium altissimum	i	f	0	0	0	0
Spergula spp.		f	0	0	0	0
Sporobolus cryptandrus	n	g	0	0	0	0
Taraxacum officinale	i	f	0	0	0	0
Thlaspi arvense	i	f	0	0	0	0
Tribulus terrestris	i	f	0	0	0	0
Trifolium repens	i	f	0	0	0	0
Urtica dioica	n,i	f	0	0	0	0
Trifolium spp.	i	f	0	0	0	0
Verbena bracteata	n	f	1	47	0	0
Vulpia microstachys	n	g	0	1	0	0
Vulpia myuros	i	g	0	0	2	0
Total species richness			53	41	52	5
Native richness			19	16	18	2
Non-indigenous richness			25	23	22	3
Unknown spp. richness			8	4	11	0

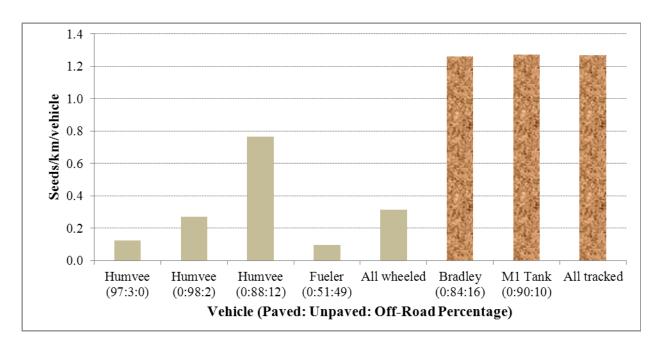


Figure 14. The number of seed that adhered to different vehicle types per kilometer driven on paved, unpaved and off-road surfaces (given as percentage) under dry conditions in June 2008 at a sagebrush steppe vegetation type at Orchard Training Area, ID. Types of wheeled vehicles did not differ significantly from each other but were significantly less than tracked vehicles. The number of seeds/km/vehicle has been corrected for the proportion of seed destroyed by the vehicle washing and transport procedure as described in Appendix A. Note that the PLS that drove the last 6 of the last 10 km off-road in a mud-bog and is not included in this graphic.

Table 5. The number of seeds gained on vehicles of different type when driven on paved, unpaved and off-road surfaces under dry conditions in June 2008 at a sagebrush steppe vegetation type at Orchard Training Area, ID. PLS = palletized loading system, Fueler = Hemtt fueler, Tank = M1 tank. Columns labeled "corrected" were adjusted for the proportion of seed destroyed by the vehicle washing and transport procedure as described in Appendix A.

Vehicle	Vehicle type	# veh- icles	Paved (Km)	Unpaved (Km)	Off- road (Km)	Total (Km)	Seed/ Vehicle	Corrected Seed/ Vehicle	Seed/ Km/ Vehicle	Corrected Seed/Km/ Vehicle
Humvee	4wd	3	2640	96	0	2736	74.0	114.4	0.1	0.1
Humvee	4wd	1	0	413	9	422	74.0	114.4	0.2	0.3
Humvee	4wd	5	0	1686	228	1914	189.8	293.5	0.5	0.8
Fueler	8-10wd	4	0	893	856	1749	27.8	42.9	0.1	0.1
PLS	8-10wd	1	0	98	6	104	2154.0	3330.7	20.7	32.0
Bradley	tracked	3	0	565	110	675	183.3	283.5	0.8	1.3
Tank	tracked	4	0	585	66	651	134.0	207.2	0.8	1.3

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5.2.2 Seed loss from vehicles

Vehicles carried seeds over two hundred kilometers under dry conditions on both paved and unpaved roads; however, under wet conditions seed loss increased dramatically. These results suggest that seeds could travel indefinitely on vehicles along roads until wet conditions are encountered, at which time they will likely be dispersed. Much higher rates of seed retention were observed under dry than wet conditions for all plate locations on both paved and unpaved roads (Figure 15). Overall, results under wet conditions were more variable than dry conditions, whereas no clear difference in variability emerged between paved and unpaved roads. All plate locations on dry paved roads retained 99% of their seeds by 256 km, whereas on dry unpaved roads, undersides and bumpers retained 96% and wheel wells 86% of their seeds by 256 km. Under wet conditions on paved roads, wheel well plates retained 0%, undersides and rear bumpers less on average ~10% whereas front bumpers retained 72% of their seeds by 256 km (Figure 15). On unpaved wet roads there were less seeds recovered with distance but the data were variable. The front and rear bumpers performed similarly, retaining approximately 95% of their seeds at 256 km, whereas the undersides retained 60% of seed, and the wheel wells 50%. Field observations suggest that, on unpaved wet roads, soil and seed are gained as well as lost. The sequence of gain and loss of seed appeared to be determined by road surface (e.g. pot-holes, puddles, corrugations) causing variability in overall retention rates.

The number of seeds retained across the different plate locations when driven over different road surfaces and conditions varied from 0 to 90 seeds (the total available) at 256 km. There was no effect of species (p = 0.54 from an F-statistic = 0.607 on 2 and 901 d.f.) nor plate surface (p = 0.90 from an F-stat of 0.017 on 1 and 295 d.f.) on seeds retained on the vehicle. There was also no effect of the speed a plate travelled on seed retention (p = 0.17 from an F-statistic = 1.78 on 2 and 297 d.f.), therefore all further analysis combined seed species and plate data, and did not account for speed. There was an effect of road surface (p = 0.0005, F-statistic = 7.774 on 2 and 297 d.f.), road condition (p < 0.00001, F = 57.4 on 2 and 299 d.f.) and plate location (p < 0.0001, F-statistic of 17.837 on 1 and 733 d.f.) on seeds retained and consequently these variables were analyzed separately. However, plate locations were grouped in a separate analysis to parameterize a retention curve for an entire vehicle.

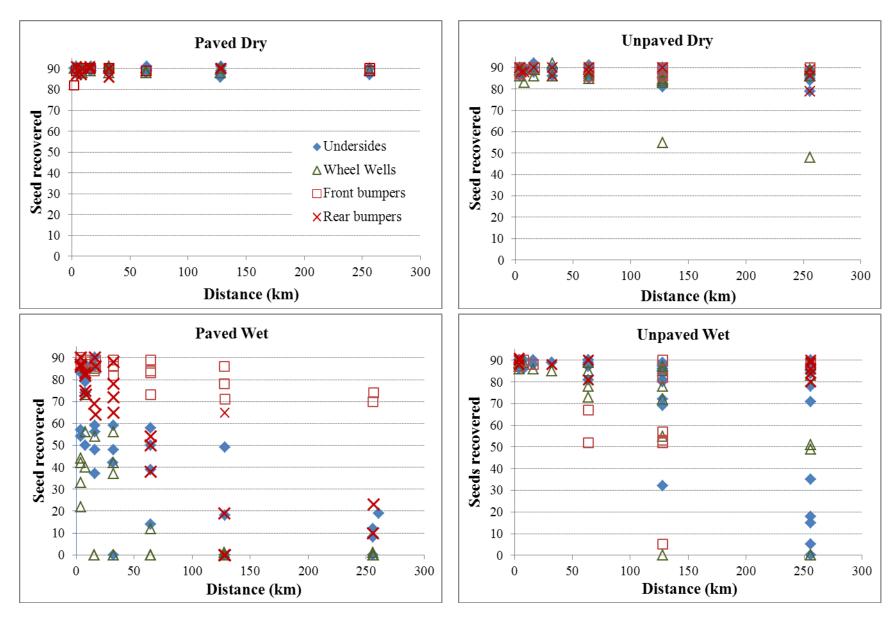


Figure 15. Number of seeds recovered from plates when driven on different combinations of road surface and condition (see graphic titles), displayed by the location of plates (see legend).

5.3 Objective 3

5.3.1 Plant species present in the seedbank versus adherence to vehicles

At Limestone Hills, 28 species were identified in the soil seedbank samples taken on the road, 5 m and 50 m from the road, with 11 unidentified. Data from the seedbank samples reveal that species richness was greater 5 m from the road (21 species) than 50 m away (16 species) and least on the road (7 species) (Table 1). Most of the species were forbs, but there were also grasses, shrubs and one tree species. The road had been treated with an oil emulsifier that may have reduced seed viability and consequently richness on the road plots. Out of the seven vegetation types surveyed, the Juniper vegetation type had the greatest soil seedbank species richness. This supports the idea that roads and edges serve as corridors for seed transport.

There were 17 species present in the wash samples and soil seedbank samples though overall more richness was observed in the wash samples. This would be expected due to the considerably larger quantities of soil recovered in the vehicle wash samples. Both vehicle wash and seedbank samples had a range of forb, grass and shrub species. Unfortunately weights and sizes of most seeds are not readily available but qualitative evaluation of information of some species suggested there was no preference for any weight or size of seed.

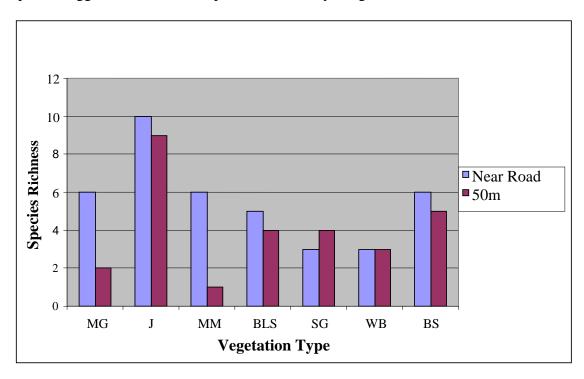


Figure 16. Species richness present in Limestone Hills soil seedbank at varying distances from unpaved roads travelling through 7 different habitat types. "Near Road" samples were taken <5m away from the road edge. "50m" = 50 meters from road edge. For Vegetation type, MG = Midgrass, J = Juniper, MM = Mountain mahogany, BLS = Black Sage, SG = Shortgrass, WB = Whitebark Pine, and BS = Big Sage as defined by vegetation layers provided courtesy of the National Guard.

At Orchard Training Area, 9 species were observed in the soil seedbank samples, of which 2 were native and 7 introduced. For the twenty-one samples taken close to the road, only 5 species were recorded (2 native and 3 non-indigenous) with *R. testiculatus* being the most abundant (Table 4). The four species observed along the transects but not along the road included the non-indigenous species *Bromus tectorum*, *Salsola kali*, *Sisymbrium officinalis* and a Boraginaceae species (these data are not shown). There were 6 species in common between the vehicle wash and soil seedbank samples, with higher species richness observed in the vehicle waste (Table 4).

Thus, the data provides confidence that a broad range of species (different grown habit etc.) adhere to the three types of vehicles assessed, with no particular bias based on seed size etc.

5.3.2 Non-indigenous plant survey

Thirty-six transects that covered a total distance of over 18 kilometers were surveyed during 2007 and 2009 for the presence and absence of NIS in the Limestone Hills training area. Additional data collected by Townsend High School students in collaboration with the National Guard Environmental Division provided valuable data to the east of Old Woman's Grave Road.

The relationships of these presence/absence data to environmental variables such as distance from roads, elevation, slope, aspect and remote imagery were modeled via logistic regression. The logistic equation using the fitted coefficient values and rasterized environmental variable data could then be used to generate probability of occurrence maps for each non-indigenous species (Appendix 2A). Model coefficients for the best models for each species are provided in Table 6.

Table 6. Coefficients from logistic regression models of six non-indigenous plant species at Limestone Hills, MT. Missing coefficient values indicate the variable did not contribute to the model based on a stepwise AIC model selection procedure.

	Alyssum desertorum	Bromus tectorum	Centaurea maculosa	Cirsium arvense	Linaria dalmatica	Verbascum thapsus
Intercept	-5.89384	7.63606	2.10221	-447.2744	-36.33253	-2.07618
Elevation (m)	0.00319	-0.00783	-0.00327	-0.02965	0.01255	
Slope (percent)	-0.04453			0.30064	0.12336	0.04003
Aspect (sine)	0.34380	0.62672	0.86314	-473.2688	11.99333	0.49631
Aspect (cosine)	0.40604		0.61733	102.14440		
Annual Radiation (kWh/m^2)		0.00149				
Distance to road (m)	0.00203	-0.00620				-0.00328
Distance to travelled road (m)	-0.00390	0.00145	-0.00357	-0.01932	-0.01177	-0.00239

A basic web application has been developed to help land managers create occupancy or probability of occurrence models and maps for species of interest. Currently DEM derived environmental variables along with proximity to road layers are provided for many states in the western USA. Managers can upload their NIS data and an occupancy map will be emailed to them shortly after. (http://ippf.msu.montana.edu).

5.4 Objective 4

Develop a NIS containment and risk management prioritization protocol to reduce the occurrence of non-indigenous plant species and the chance of propagules being transported between and within installations in the continental U.S. (CONUS) and outside CONUS e.g. overseas deployment (OCONUS) based on the results from Objectives 1-3.

The following general guidelines provide a series of steps that should be followed in order to minimize the risk of transporting NIS, 1) from installations that have a NIS problem to installations which are presently not invaded or have NIS at low abundance, and 2) from NIS invaded areas to non-invaded areas within the same installation. While these steps are necessarily general, they could easily be adapted to suit the needs and considerations of specific installations. There is a general and specific main task area list. The first set of tasks should be completed as general practice *i.e.* regardless of what types of exercises/activities are occurring. These tasks are meant to form a baseline of knowledge related to the current status and distribution of NIS, as well as an understanding of the types and effectiveness of control measures being used within the installation. The second is a set of tasks that should be completed prior to specific exercises or activities in order to minimize the risk of unwanted NIS propagule movement within or between installations.

1. General Procedural Tasks NIS Containment and Risk Management

Know your potential problem species

- What are your land management goals (e.g. maneuverability of vehicles, cattle grazing)?
- Define the NIS of most concern to your goals by making a list of:
 - o designated "noxious" weeds and undesirable species in your state
 - o designated "noxious" weeds and undesirable species in close proximity to your area
 - o designated "noxious" weeds and undesirable species in your installation

Determine NIS risk

- Refine your NIS list to a target list of those NIS that will be monitored and managed
- Establish management objectives for each target NIS (e.g. eradicate, reduce density or area, establish native species)
- Decide on management practice to achieve NIS objective
- Prioritize highest occurrence NIS areas using prevention tools

Prevention tools for NIS

- Survey NIS to determine spatial distribution on the landscape
- Use spatial NIS data to create an occupancy or "risk" map
- Monitor select populations to assess effectiveness of control practices (e.g. assess cover or density before and after treatment, plus controls)
- Use data from bullets above to help prioritize areas to concentrate on when resources are low
- Continually learn from your approaches and incorporate adaptive management practices
- See ippf.msu.montana.edu * for more details of how to achieve these steps

• Wash vehicles before entering and when leaving installation, or after moving through high propagule area

* Currently set up for some Western States. Contact lrew@montana.edu if you are interested in other areas.

2. Specific Exercise or Other Activities Tasks to Reduce or Contain NIS

Know the types of vehicle accessing site

- What type of vehicles are entering sites (e.g. tracked, tactical wheeled, civilian pattern, ATV)?
- What are the vehicles typically used for (e.g. military exercises, road maintenance, agriculture, hunting)?
- Where are the vehicles likely to have been driven prior to accessing the site (off-road, unpaved, paved, installation, area of country etc.)?
- Where are the vehicles likely to be driven when on site (off-road, trails, unpaved, paved)?
- What are the current and forecasted moisture conditions (wet or dry)?

Vehicle risk level assessment

- Vehicle type:
 - o tracked vehicles gain more seeds than wheeled vehicles
 - o within wheeled vehicles those with low and more featured chassis (e.g. Humvees, ATVs) are likely to gain and spread more seed
- Vehicle usage: vehicles travelling off-road, earth moving etc. likely gain more propagules than those travelling on paved roads
- Road surface: Vehicles travelling off-road gain more seed than, in decreasing order, those driven on trails, unpaved roads, and paved roads
- Road conditions:
 - o more propagules are gained on unpaved roads under wet than dry conditions
 - o more seed is lost on wet paved roads than wet unpaved roads or dry paved/unpaved roads
- List your vehicles in order of risk of transporting NIS

Prevention tools for vehicles

- Use NIS risk maps to highlight areas with high risk (occupancy) for different target species
- Avoid driving through high risk areas particularly under wet conditions or before entering pristine areas
- Wash vehicles prior to entering and departing from the installation in order of risk as defined by vehicle risk assessment above
- Contain the soil waste once it is off of the vehicles seal in containers (e.g. double bag the waste) and place in landfill type environment
- Filters (~200 micron) should be placed on hose ends that are releasing gray water to prevent seed escape
- Construction of new roads, and maintenance of current roads (grading, deicing, etc.) should be performed with gravel from weed-free gravel pits

These tasks and steps apply to CONUS installations but could also be applied to OCONUS environments. However, a general overview and procedures for OCONUS movement of vehicles with regard to NIS is addressed by ERDC/EL TR-07-8 report (Cofrancesco *et al.*, 2007) published after the commencement of this project.

5.5 Objective 5

Evaluate and recommend improvements to the operation and design of the different vehicle washing units to improve their effectiveness, efficiency and containment capability. Develop specifications/classifications and standards for vehicle washing systems that are intended for remote and portable applications. (Joint USDA Forest Service SDTDC phase of the project).

Results of the efficiency of the different wash units are detailed under Objective 1 (Section 5.1.1 above) and it was intended that as a result of these experiments and a few others detailed in (Fleming 2008) that we would be able to make recommendations on improvements to the operation and design of different vehicle was units to improve their effectiveness, efficiency and containment capability. The conclusions of the Forest Service (Fleming 2008: http://www.fs.fed.us/t-d/pubs/pdf/hi_res/08511809hi.pdf) are provided here with more salient points *highlighted*:

"we tested five systems that we considered to be representative of a wide range of equipment, we did not find any single mechanical feature that clearly ensured an effective system. We consider the presence of an undercarriage wash system to be advantageous, though we have to state that the unit which did not have them performed similarly to the rest. Therefore we do not have any data to support the specification of any particular piece of equipment or combination.

As we had to measure all the soil waste recovered from each vehicle wash unit and then measure what they had missed we could not specifically address how, under normal field conditions, each unit contained the waste. However, it was apparent that some contractors were much more prepared and concerned with reducing contamination at the site than others. The best approach was one where the soil waste was double bagged. This approach ensured that no waste could be left to contaminate the areas. In addition the double plastic bagging meant that if the soil waste was left for a period of time (> a few days) many of the seeds were killed.

In some regions the Forest Service had already adopted system standards and practices based in part on the results of this test. The agency is assuming responsibility for final disposal of all solid waste to ensure that the primary objective of containing invasive species is met. All tests systems recycled the water after filtration, and we recommend that fresh water will be provided by the agency in an effort to help reduce the contract cost.

Recommendations

We should as agencies of the Federal Government:

1. Develop minimum equipment parameters and performance standards for washing systems which we contract.

- 2. Define washing systems by type with regard for water and waste containment, spray system, process rate, and cost range. We are still considering that there may be a cost advantage to systems that do not recycle the wash water but we do not yet have a definition of this type of system and we do not have any comparative data on the efficacy, productivity, and cost of these systems. We recommend formal comparative testing of the presumably less costly type 2 systems mentioned in the main part of the report.
- 3. Establish simple, easily followed test procedures to ensure that our minimum requirements are met by measureable repeatable criteria.
- 4. Convert our contracting practices to a performance basis so that overall cost, and deployment time are all factored into the decision process. The resulting choice would represent the best value available.
- 5. Define specific acceptable guidelines for waste disposal that are universally acceptable. Some areas may allow variances but at least we would have a worst case set of practices that contractors and contracting officers could revert to when there is no clear statute or rule governing disposal methods and practices. In any case, we should at least define a particle size that all wastewater and sludge will be filtered to before disposal.
- 6. Establish a wash time of at least 6-9 minutes per vehicle to ensure that the majority (60-90 percent) of available soil debris is removed, given a typical two-person crew with two high pressure spray wants, and preferably with undercarriage spray systems. These data are generated from two different site conditions."

All five of the wash units we evaluated in a controlled field experiment (Objective 1) removed the majority of the soil and other matter, although they left considerable amounts (on average about 23%) on the vehicles. The results of the trial in Ione showed that there was no significant difference among the wash systems, despite the differences in water volume and pressure. There was a trend for more waste to be removed from the fire engines than the trucks when washed for the same duration (5 minutes). The operators attributed this to the more complex underbody construction of the 4x4 trucks as compared to the larger, smooth surfaces of the fire engines. The same pattern was observed in Objective 2 with more seeds tending to adhere to Humvees than tactical wheeled vehicles. More material was washed off the bulldozers than the other vehicles; albeit these vehicles were washed for a longer time period for both Objective 1 and 2.

As a result of our continued experience with mobile vehicle wash units following on from the study in Ione *we now recommend a more specific set of wash times* than the original Forest Service report (Fleming 2008). The recommended wash times are provided in Table 7.

Table 7. Recommended vehicle wash times in minutes using a mobile wash unit.

Condition	Civilian Pattern	Tactical	Tracked
Condition	4x4WD	6-10 wheel	
Dry	6	12	18
Wet	12	18	36

It would appear that these recommendations are similar to times used by the military although from our communication with Dr. Cofrancesco of ERDC EL and Dr. Peter Egan and LTC Jamie Blow of the AFPMB, we must reiterate that there is no standard time for military washing. LTC Blow stated in a 06 Oct 08 e-mail: "There is no standard washing time for any type of vehicle. Vehicles are washed until they pass the standard however long this may take. This may vary based on the number of operational power washers available for use, the number of personnel, and the number of wash racks. I am sorry I can't be more specific."

The one criterion which appears to apply is stated within UFC 4-214-03, 16 January 2004, Unified Facilities Criteria (UFC); Central Vehicle Wash Facilities. This document is used to plan for the construction of new wash facilities, and is the DoD standard for this purpose. The washing rate, or throughput, is mentioned in two locations (*with our highlighting*):

On Page 3-6, under the heading "Prewash," the following is stated: "Process Rate. In a tracked bath lane, *six to ten tracked vehicles per hour can be washed*. In a dual-purpose lane, ten to fifteen wheeled vehicles per hour can be washed. The amount of soiling will determine the actual number of vehicles that can be processed through the facility; the heavier the soiling, the slower the vehicles can be processed."

Further, on Page 3-7, under the heading "Wash Stations," the following is given: "Sizing with a prewash. When a bath prewash is provided, the number of wash stations should be rounded. If the maximum washing time is critical, between two and five per tracked vehicle bath lane. A process rate of 3 to 6 vehicles per hour at each wash station can be expected after the vehicles have been washed in the bath. Since all vehicles will not go through the prewash bath, calculations of lanes must account for longer wash times for these vehicles. A process rate of 2 to 4 vehicles per hour for large, odd shaped, or tandem units can be expected. A process rate of 4 to 10 vehicles per hour for small wheeled vehicles such as jeeps or ½ tons can be expected. Installations with a limited washing time or a large percentage of wheeled vehicles to wash will require more wash stations. In any case, the number of stations can be calculated using the processing rates for each type vehicle and its type.

In the next paragraph, the following is stated: "Sizing without a prewash. The process rate for vehicles at the wash stations will depend on several factors, but usually will be between 1 and 10 vehicles per hour".

It should be noted that our recommended wash times (Table 7) correspond fairly well to the estimated military wash rates. We washed wheeled vehicles for between 4-9 minutes, tactical wheeled for 8.5-13 minutes and tracked vehicles for 13-18 minutes under dry field conditions. These durations were doubled under wet conditions. In our discussion with the 1-163rd Infantry Battalion at Orchard Training Area we found that their regular washes at the OTA washing facility took anything from 10 minutes to several hours depending on the vehicle and where it had been. The military personnel cooperating with our washing trial were delighted to be involved because they stated the commercial wash unit did a much better job with much shorter wash times than they could achieve. They stated that *the undercarriage washers and the pressure hoses did a much better job than the non-directional low water pressure hoses they were using*. Thus, please refer to Table 7 for our recommended wash times.

6.0 Conclusions and Implications for Future Research

6.1 Conclusions

This project has enabled us to quantify the potential of different types of vehicles (tracked, tactical wheeled and civilian pattern) to transport plant propagules (mostly seeds) providing us with an original, unique and valuable data set. While everyone has suspected that vehicles transport NIS seeds, there was little empirical evidence from controlled experiments until this study was conducted. The evaluation of number and abundance of species transported as part of normal military exercises has shown that even during the summer, before the current season's seeds have been shed, seeds and other matter do adhere to, and are transported by, vehicles driven on combinations of paved, unpaved and off road. Results from the vehicle seed loss experiment showed that under dry conditions seeds can be transported by a vehicle for more than 250 km on a paved or unpaved road. However, under wet conditions, up to 100 percent of seeds can be lost from the vehicle chassis on both road surface types.

In this project we created non-indigenous species occupancy or "risk" maps from survey data taken at the site (see Appendix B). Such maps could be used to help direct training exercise locations – for example areas with a high risk of undesirable species could be avoided or at least not driven through before entering more pristine areas or under wet conditions – particularly if it is not possible to wash vehicles in between. The effectiveness of such protocols could be assessed by survey and monitoring of species in these areas and determining change over time. Occupancy maps can also be used to prioritize which areas are most at risk and should be prioritized for management. Such data can also be combined with NIS control data, and potential risk of spread and establishment, to obtain a better understanding of the effectiveness of current management practices in achieving their goal of reducing NIS abundance and spread.

Our recommendations would be that new facilities invest in undercarriage washers and high pressure hoses rather than low pressure hoses. From our experiments and field washing experience with a representative mobile wash unit we would recommend the most efficacious wash durations should be approximately 6 minutes for civilian pattern vehicles, 12 minutes for tactical wheeled, and 18 minutes for tracked, all under dry conditions. Considering that wheeled vehicles and tracked vehicles picked up 21 and 46 times more seed respectively under wet conditions we would recommend from field experiences and studies that wash times be increased to at least 12, 18 and 36 minutes for civilian pattern, tactical wheeled and tracked vehicles respectively.

6.2 Implications for Future Research

We achieved all of the project objectives and have a few suggestions of where there are still gaps remaining and/or where future research could progress. While the larger scale field studies with the National Guard provided useful original data, more specific and replicated information are required on seed gain and loss rates under a large range of field conditions and vehicle types. Thus, we would recommend the development of the type of retention frame and plate system we created to obtain replicated and accurate results. Such data could be used to develop spread maps of propagules from particular areas under different driving surfaces and conditions for varied

vehicle types. Combining these data with population dynamic models parameterized for different environments generated through occupancy models would provide greater utility of this predictive simulation modeling approach. We have started to develop such a model for wheeled vehicles under the conditions we evaluated and would eventually like to integrate it into the prioritization protocol mentioned below to help reduce NIS spread. Thus, we would recommend further exploration of predictive simulation modeling of seed spread and establishment.

A comparison and evaluation of central vehicle wash facilities (CVWF) and newer higher volume wash units that were not developed at the time of this study (e.g. http://rrmobileservices.com/janda/inner.php?PageID=11) with the type of mobile wash units we used would be very beneficial in order to be able to make more valid comparisons and more stringent recommendations of wash times for different vehicle types driven in varied conditions. This should be combined with education on the importance of vehicle washing as an invasive plant species prevention tool to increase the likelihood that washing protocols are adhered to.

7.0 Literature Cited

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Appendix A: Supporting data on the effect of varying storage conditions on seed germination

Methods

The experiments related to quantifying seed transport by vehicles required the transport of soil and seed material from off-site locations to MSU where the mix was reported for germination and growth in the greenhouse. Therefore, to determine the best storage method to use for future transportation of soil and seeds from field locations back to MSU, and to evaluate the proportion of seeds destroyed by this process we performed some preliminary experiments.

The effects of cold storage and air dry treatments on the germination of agricultural species of varying seed size were evaluated first. Agricultural species: field oats (Avena sativa), mustard (Sinapis alba), and cultivated flax (Linum usitatissimum) were used to minimize the effect of varying seed viability. Each sample consisted of a homogenized mixture of 500 grams of a pasteurized loam soil, 30 seeds of each above stated species, and 300 ml of water. There were three different initial temperature regimes, 15.5 °C, 29 °C, 43 °C, that were chosen to represent possible field conditions. Samples were left at these temperatures for 0, 2, 4, 8, 16, and 32 hrs, again, trying to represent possible timeframe that the soil and seed mixture would be suspended in the vehicle wash unit. After the initial temperature and hour treatment, the samples were assigned to one of two storage treatments (air dry or cold storage), or a control group. Air dried samples were placed in 10 cm x 15 cm trays to dry in the greenhouse while the cold storage samples were stored in closed containers at 4°C. Both sets of treatment samples were left for 96 hrs. The cold storage samples were then placed in trays and all the samples (i.e. air dry and cold store) were watered. Controls were placed in trays and watered immediately following the initial temperature and hour treatments. Seedlings were recorded and removed daily. There were a total of four replications for each treatment regime, and the whole experiment was replicated twice. Percent germination was used to determine the effects of the treatments. These values were corrected for inherent seed viability as tested using a subsample of 90 seeds of each species placed on wetted blotting paper and left to grow in a controlled environment chamber. To determine if there were any differences in germination rates between treatments, data for each species were analyzed using an ANOVA with a Tukey Correction for Multiple Comparisons.

A second experiment allowed us to estimate the number of seeds that were destroyed due to the storage and containment procedures that directly replicated the conditions that samples were subjected to before and during transportation from Ione, CA and our Objective 2 field sites. Due to logistical constraints, the cold storage treatment was used in the field. Nine species were used, including: slender wheatgrass (*Agropyron trachycaulum*), field oats, purple coneflower (*Echinacea purpurea*), cultivated buckwheat (*Fagopyrum sagittatum*), kochia (*Kochia scoparia*), cultivated flax, yellow sweet-clover (*Melilotus officinalis*), Kentucky bluegrass (*Poa pratensis*), and mustard. These species were chosen to represent a broad range of seed shapes and sizes, oats being the largest and kochia the smallest. Thirty seeds from each of the selected species were incorporated into 500 g of Montana State University loam soil, and mixed with 300 ml of water: the experiment was replicated four times. These samples were placed in a controlled environment chamber at two temperatures to represent the average day (30 °C for 7.5 hrs) and night temperature (12 °C for 12 hrs) of the field conditions, for 19.5 hrs total. No light was used

because the samples were contained in opaque tanks while they were settling in the field study. The samples were then placed in coolers (~0.09 m³) with six ice blocks for 13.5 hrs while keeping the coolers at room temperature (15 °C). The lid of the cooler was then removed and the coolers were placed in cold storage (4 °C) for an additional 57 hours. This procedure took a total of 90 hrs. This procedure closely replicated transportation conditions during the Ione, Limestone Hills and Orchard Training Area field studies. Control soil and seed mixes were potted at the same time as the treated samples. The samples for Ione were assessed and recorded for germination for 84 days or until no further germination occurred, (samples from Limestone Hills and Orchard Training Area field studies were kept for 18 months). To determine if there were any differences in germination rates or totals between treatments, data for each species were analyzed using an ANOVA with Tukey Correction for Multiple Comparisons.

Results and Discussion

The overall trends showed that percent germination was generally higher at 15.5 °C than 29 °C or 43 °C treatments, and oats germinated better than mustard or flax. As would be expected, the number of seeds germinating decreased with time under each treatment for all species. Considerable analyses of the data were performed but because we used the cold storage method to transport the soil and seed mix, and as the waste material was contained in the weed wash units for approximately 16 hrs for all field trials, only the results of those treatments will be described here. Generally there was no difference in germination between the 15.5 °C and 29 °C (1 of 6 – see Table A1), whereas the 15.5 °C and 29 °C treatment produced more germinated seeds of all species than the 43 °C (with one exception: experiment 1, flax 29 – 43 °C treatment). The control, air dry and cold storage treatment did not differ in overall germination half of the time, and the cold and control treatment did not differ for 4 of 6 comparisons (Table A1i).

Table A1. Statistical differences in the total germination of flax, mustard and oat seed stored at 15.5, 29 or 43 °C for 16 hours, prior to being air-dried (at 15.5 °C) or cold-stored (4 °C) for 96 hrs prior to be placed in flats in the greenhouse and watered daily. Control samples were placed in the greenhouse after the 16 hr temperature treatment. Experiments were analyzed separately due to significant differences at that level. Data were transformed for normality, analyzed with analysis of variance and Tukey's Comparison of Multiple Comparisons performed. The values provided are from Tukey's test, except where there was no difference at the storage treatment level as a result cells are merged and labeled NS represent no significant difference. P values of less than p<0.001 have been summarized as such, otherwise the actual value was provided. vs. = versus.

		Treatment comparisons						
Experiment	Species	Temperature			Storage			
		15.5 vs. 29 °C	15.5 vs. 43°C	29 vs. 43°C	Control vs. air	Control vs. cold	Air vs. cold	
1	Flax	p<0.001	p<0.001	NS	NS			
	Mustard	NS	p=0.013	p=0.014	NS			
	Oats	NS	p<0.001	p<0.001	NS	NS	p=0.033	
2	Flax	NS	p=0.009	p=0.001	NS	p<0.001	p<0.001	
	Mustard	NS	p<0.001	p<0.001		NS		
	Oats	NS	p<0.001	p=0.001	NS	p=0.04	p=0.011	

Evaluation of the cold treatment (96 hours stored at 4°C) replicating the transportation schedule from the field sites to MSU and the control treatment (immediate placement in greenhouse conditions) showed that the number of seed germinating differed between species and under control and treated conditions (Figure A1). Flax and sweet clover had significantly lower germination than the other species but these differences were not statistically related to seed size or seed weight in this experiment. No germination pattern was observed by seed size or seed shape and the average transport germination was 45.4 %. 1-45.4 was used as a conservative correction factor to estimate total number of seeds that adhered to vehicles as part of Objective 2.

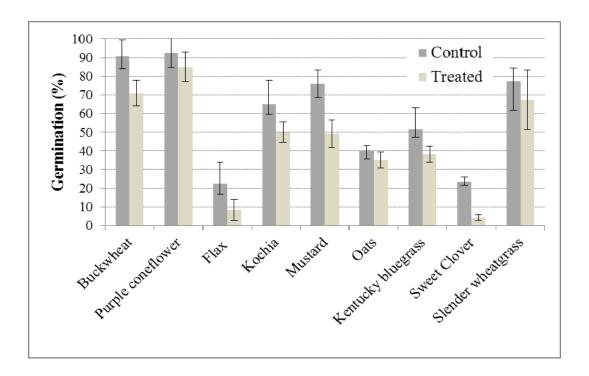


Figure A1 Percentage of germinated and emerged seeds for nine selected species under controlled and treated conditions. The species were chosen to represent a range of seed shapes and sizes. The transport treatment replicated conditions of seeds transported from the field to the MSU greenhouse for Objective 1 and 2, and were used to correct the germination data for Objective 1 and estimates of seed number for Objective 2. Treated = 96 hours stored at 4°C, control = immediate placement in greenhouse.

Appendix B: Example of risk maps produced from NIS occurrence data

Maps were created using data from transects completed in the Limestone Hills Training Area by the Montana State University crew. Specifically, these transects were in areas surrounding the Main Access Road and Old Woman's Grave Road. Additional data, collected by high school students from Townsend in collaboration with the National Guard Environmental Division, in flatter areas to the east of Old Woman's Grave Road, were also used to create these occupancy maps.

Occupancy maps were created for *Alyssum desertorum* (desert alyssum), *Bromus tectorum* (downy brome or cheatgrass), *Centaurea maculosa* (spotted knapweed), *Cirsium arvense* (Canada thistle), *Linaria dalmatica* (Dalmation toadflax), and *Verbascum thapsus* (common mullein). There are two maps for *A. desertorum*; the first map details the occupancy predictions with the 2007 and 2009 exercise tracks overlaid, whereas in the second map, tracks are removed for improved visual clarity. Maps for subsequent species do not have the exercise tracks overlaid, as they were identical for each species. The maps for both *C. arvense* and *L. dalmatica* have enlarged insets as they were predicted to be at low density.

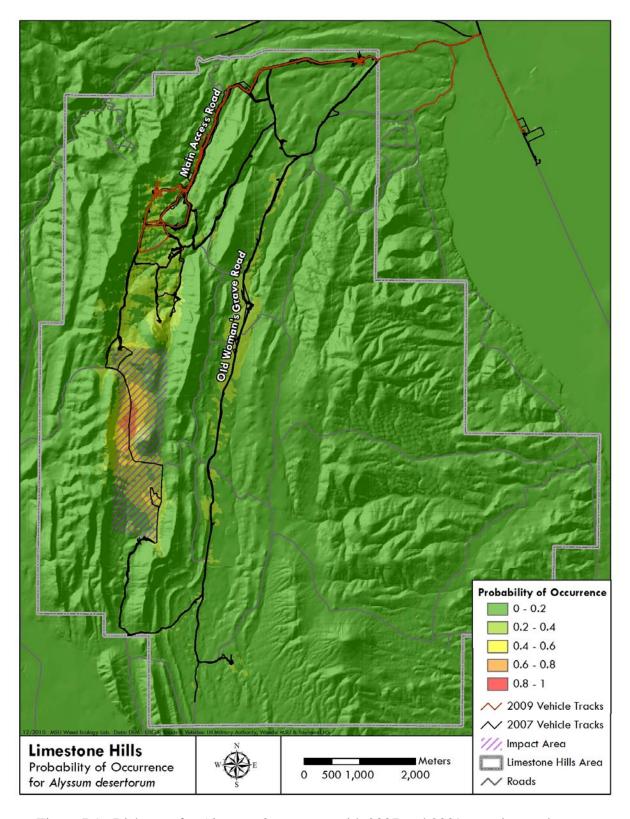


Figure B1. Risk map for *Alyssum desertorum* with 2007 and 2009 exercise tracks overlaid.

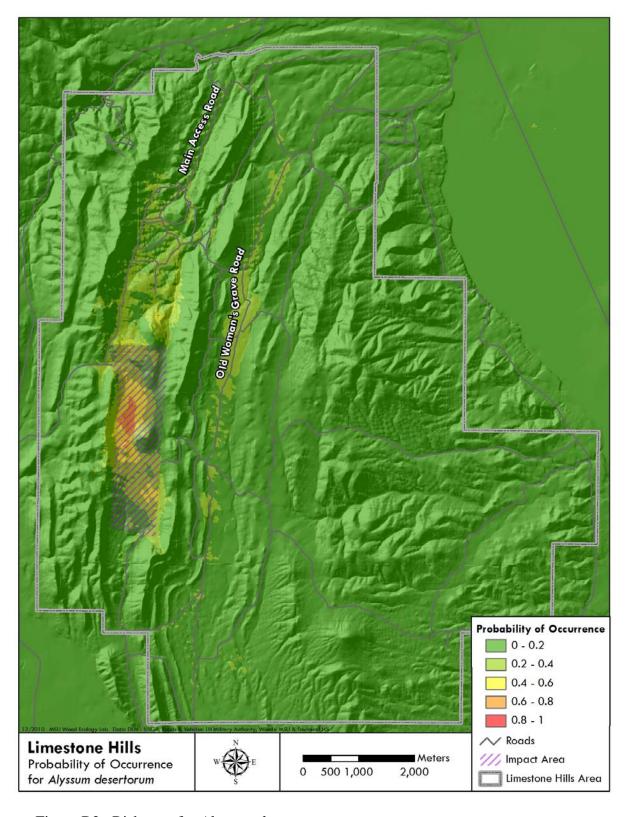


Figure B2. Risk map for Alyssum desertorum.

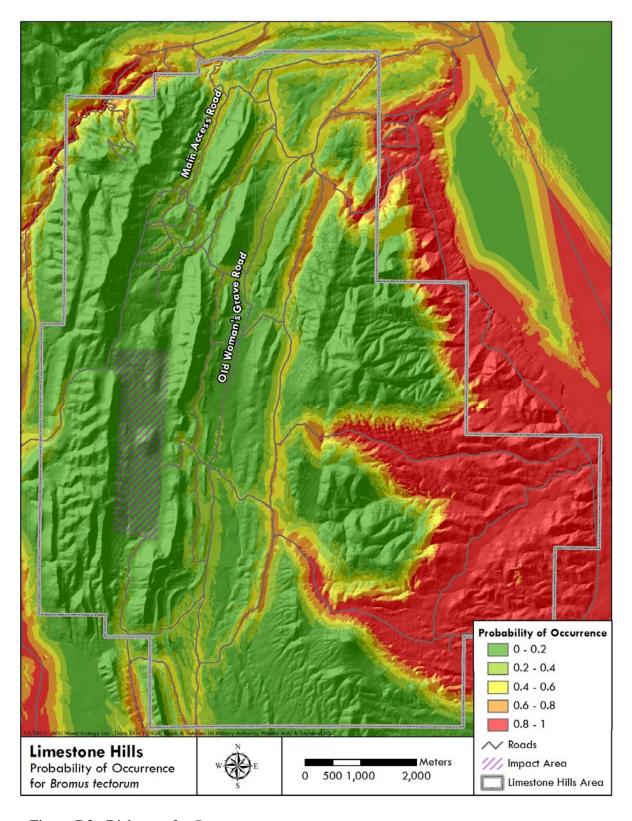


Figure B3. Risk map for *Bromus tectorum*.

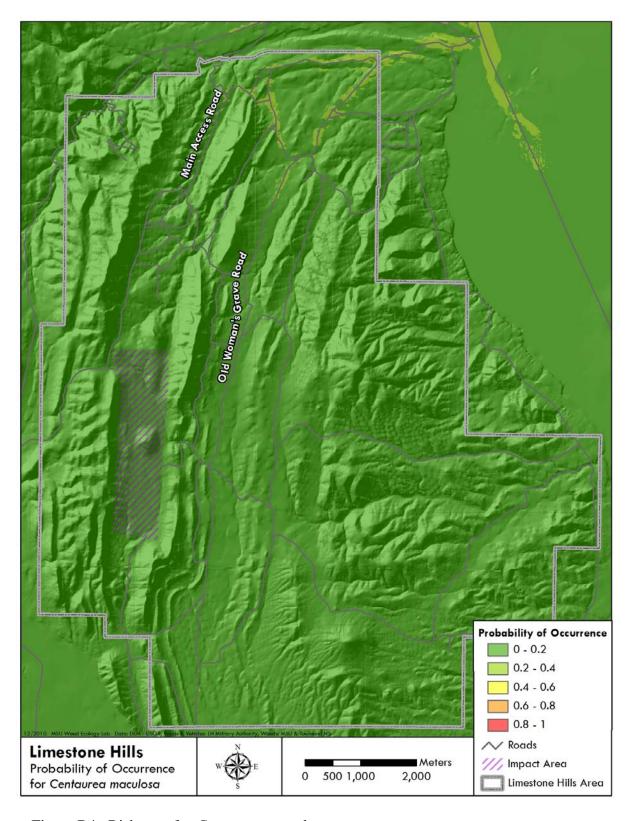


Figure B4. Risk map for Centaurea maculosa.

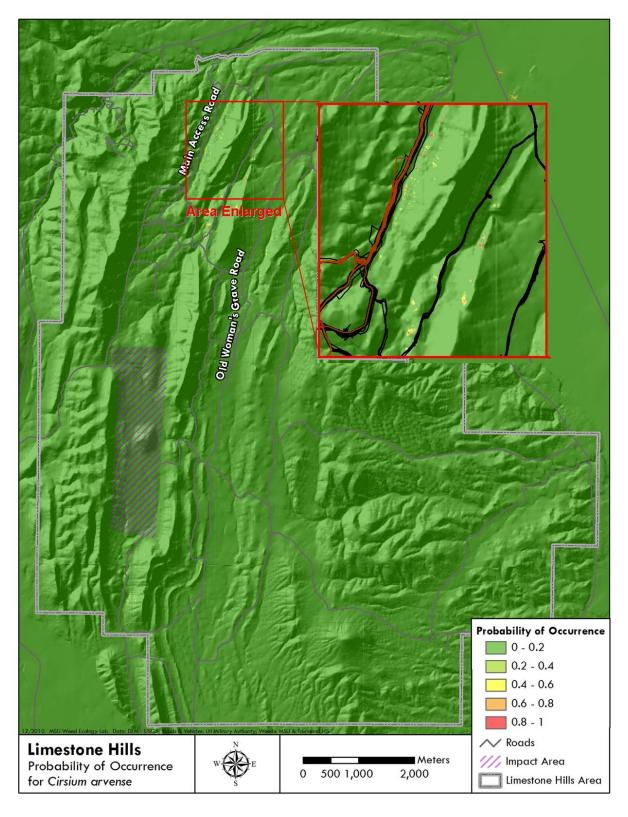


Figure B5. Risk map for *Cirsium arvense*. Since this species had low probability of occurrence, an enlarged inset is included in this figure.

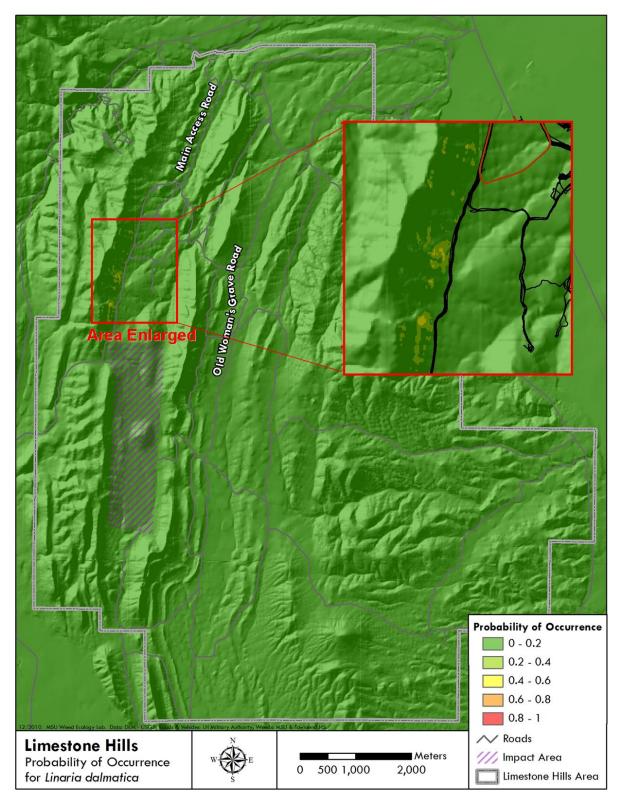


Figure B6. Risk map for *Linaria dalmatica*. Since this species had low probability of occurrence, an enlarged inset is included in this figure.

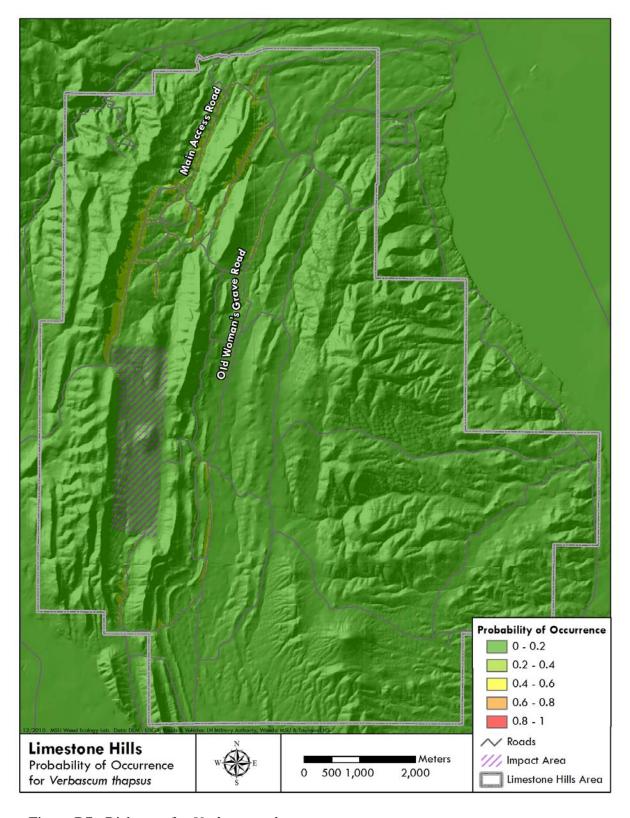


Figure B7. Risk map for Verbascum thapsus.

Appendix C: List of Scientific/Technical Publications

Journal articles

- Taylor K, Brummer T, Wing A, Taper M, Rew LJ, (*in preparation*). Human mediated seed dispersal: an empirical evaluation of long distance dispersal by vehicles. *Diversity and Distributions*.
- Rew LJ, Pollnac F, Brummer T and Balbach H. (*in preparation*). Evaluating the potential of plant propagule movement by vehicles. *Frontiers in Ecology*.
- Rew LJ, Pollnac F, Brummer T, and Maxwell BD. (*in preparation*). Quantifying and predicting seed dispersal from vehicles. *J. Applied Ecology*.

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